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Radioimmunoassay and related techniques to improve artificial insemination programmes for cattle reared under tropical and sub-tropical conditions

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FOREWORD

The IAEA and the FAO, through the activities of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and their technical co-operation (TC) programmes, support isotope-aided research into methods for improving animal productivity in developing countries. This has focused on animal reproduction and nutrition, with emphasis on local small-farm production systems, and has led to the identification and subsequent alleviation of a number of problems related to the reproductive management and feeding of ruminant livestock.

Artificial insemination (AI) is widely used for improvement of cattle production in developed countries. Its use in developing countries is less widespread and the results obtained are far from satisfactory. Under tropical small-farm conditions, a number of socio-economic, organizational, biological and technical factors make the service more difficult to provide and also less efficient. If the major constraints can be identified and overcome, this technology would become more widely adopted and contribute to an increased production of milk and meat, leading to better food security and poverty alleviation.

The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture therefore convened a consultants meeting in May 1994 to advise on the applicability of radioimmunoassay (RIA) for measuring progesterone in milk of dairy cattle to identify the major causes of conception failure and reproductive wastage when AI is used under the conditions prevailing in developing countries. The consultants recommended the initiation of a co-ordinated research project (CRP) on this topic, and developed a comprehensive technical document including the sampling protocol and the range of information that needs to be recorded in order to obtain conclusive results. A five year CRP on the “Use of RIA and Related Techniques to Identify Ways of Improving Artificial Insemination Programmes for Cattle Reared Under Tropical and Sub-Tropical Conditions” was initiated in early 1995.

The CRP resulted in the development and standardization of methodologies and protocols, including the computer software program termed AIDA (Artificial Insemination Database Application), to determine current status and identify constraints. These methodologies and protocols are now being applied on a wider scale in Member States through regional TC projects in Asia and Africa and country TC projects in Latin America. Contributing to the wider application of progesterone RIA for field level problem solving and provision of diagnostic services of direct benefit to farmers was the completion of a novel “self-coating” RIA system, based on a monoclonal antibody to progesterone, at the FAO/IAEA Agriculture and Biotechnology Laboratory at Seibersdorf. This RIA system dramatically reduces the cost of assaying milk samples and facilitates the development of capability to produce the essential reagents in selected national laboratories of Member States. Training and infrastructure development to achieve self-sufficiency in RIA requirements within each geographical region and to promote the sustainability of RIA applications in livestock production are being pursued through regional and individual TC projects.

The FAO and the IAEA wish to acknowledge the valuable contributions made by all participants to the successful completion of this project. The IAEA officer responsible for this publication was O. Perera of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

CONTENTS

SUMMARY OF THE CO-ORDINATED RESEARCH PROJECT ON RADIOIMMUNOASSAY AND RELATED TECHNIQUES TO IMPROVE ARTIFICIAL INSEMINATION PROGRAMMES FOR CATTLE REARED UNDER TROPICAL AND SUB-TROPICAL CONDITIONS

1. BACKGROUND

1.1. Scientific considerations

Artificial insemination (AI) is the oldest, but is still the most widely used, of the "biotechnologies" which are currently being applied for improvement of livestock production in the world. It is used in both developed and developing countries, particularly within the cattle industry, for rapid genetic improvement. However, there are several socio-economic, organizational and biological factors which affect the efficiency of this technique, reducing the success rate and preventing it from being used by a larger proportion of farmers. If these constraints can be overcome, not only would the current users of AI services benefit, but the technology would also become more widely adopted.

Any attempt to improve the efficiency of AI has to be based on an understanding of the most important causes for inefficiency. The traditional methods used for this rely on recording and analysis of reproductive events such as oestrus, services, pregnancies and calvings. However, these records are often inaccurate and do not allow an assessment of the importance of factors such as efficiency and precision of oestrus detection by the farmers and incorrect timing of insemination. The information required for more accurate assessment can be obtained by combining traditional methods with the measurement of a key reproductive hormone, progesterone, in samples of milk or blood collected at strategic times in relation to the reproductive cycle of the animal.

Progesterone is produced by a transient organ which develops in the ovary after ovulation, called the corpus luteum (CL). The CL functions for a specific period and, if conception does not occur, undergoes regression. If conception does occur, however, it continues to function and to secrete progesterone throughout the period of gestation. Thus the concentration of progesterone in body fluids vary according to the reproductive status of the animal.

When measured in samples collected at appropriate times in relation to AI, progesterone values can be used in conjunction with related physiological data to evaluate more precisely the causes for poor reproductive performance. For example, it has been reported that in some herds showing poor fertility, 15–20 % of the cows are inseminated when they have elevated progesterone levels indicative of a non-oestrous state. Also, low progesterone concentrations 22–24 days post-insemination indicate that the cow is not pregnant and, if not observed returning to oestrus, indicates poor oestrous detection.

1.2. Consultants meeting

In May 1994, the Joint FAO/IAEA Division held a consultants meeting to advise on the feasibility of using the above approach to monitor and improve the performance of AI services in developing countries. The meeting concluded that there was ample justification for the initiation of a Joint FAO/IAEA Co-ordinated Research Project (CRP) to study these aspects and that, subsequently, a simple RIA laboratory for measuring progesterone established in AI centres or in milk collecting centres operated by State or farmer organizations could provide valuable information for evaluating the current performance of AI services, identifying and rectifying constraints that may limit their success, and instituting services for farmers to improve their reproductive management.

The meeting discussed the basic requirements for participation in the programme as well as the experimental design and the information to be obtained. It recommended that the solid-phase radioimmunoassay (RIA) method based on antibody-coated-tubes and ^{125}I-progesterone was the most appropriate technique for use in this CRP, due to its robust and reliable operation in developing countries. The use of enzyme-based techniques, whether laboratory-based or cow-side, were considered inappropriate at the present time due to their lack of consistency and high cost. The scientific details of sampling protocols and assay procedures as well as guidelines for interpretation of the results were discussed and documented to assist participants in the implementation of the project.

2. OBJECTIVES AND SCOPE OF THE PROJECT

2.1. Objectives

The main objective of the CRP was to improve the quality of AI services in developing countries through the identification of causes of inefficiency and to implement appropriate changes to rectify them. The programme was expected to serve as a vehicle for instituting better training and professional development of AI technicians, as well as for educating farmers on the importance of oestrous detection and improved husbandry practices.

These objectives were expected to be achieved by:

- Improving the efficiency of oestrous detection, resulting in increased numbers of animals being inseminated;
- Improving the timing of insemination, resulting in increased conception rates;
- Identifying anoestrous animals at time of expected breeding, allowing adoption of appropriate remedial measures; and
- Identifying animals which should return to oestrus after AI, focussing attention of farmers to detect them and have them bred in time.

2.2. Scope

The programme was focused mainly on dairy and dual-purpose cattle (with beef cattle included where relevant) and the target farms were smallholder farms in Asia and medium to large farms in Latin America. It was emphasized that the farms should be representative of the specific area selected for study.

It was envisaged that the programme would last five years. Participants in the programme were expected to initially conduct a survey to establish the current status of fertility in herds subject to AI, and to identify the constraints limiting performance at each location. This phase was expected to take 2 years and to cover 400–500 first inseminations. Subsequently, participants would focus their studies on one or more of the constraints or limitations identified and institute improvements and/or interventions. These could include one or more of the following: improving efficiency of oestrous detection; more accurate timing of AI; changing management and feeding practices; improving the quality and handling of semen; improving the technique of AI technicians; and appropriate recording and maintenance of data related to AI. Some of these activities may be implemented through approaches such as training workshops for AI technicians, seminars and group discussions involving farmers and farmer organisations. The interventions would be evaluated during and after implementation in order to assess the success of the programme.

It was anticipated that the chief scientific investigator (CSI) of each research contract would be a scientist from either a university or government research institute. He or she should have access to a collaborating organization which undertakes relatively large scale AI on a routine basis (with over 10 000 AI/year) These could be AI centres or AI services operated by the relevant government ministry or state institutes, farmer cooperatives or associations. Preference would be given to those having access to on-going FAO supported projects and/or other bilateral projects on AI. At least one of the additional scientific staff should be a senior supervisory officer in the AI services of the collaborating institute. This was considered essential in order to ensure continuity and sustainability after the completion of the CRP.

3. IMPLEMENTATION

A project document containing detailed information on the CRP, including scientific background, study design and all technical aspects of sampling, progesterone assay and data management was prepared and circulated to institutes in FAO/IAEA Member States. The CRP was also advertised in the bi-annual Newsletter of the Animal Production and Health Section.

Proposals for research contracts were received from some 30 institutes, mainly in Asia and Latin America. These were technically evaluated and 14 (7 in Asia and 7 in Latin America) were selected for award of contracts. In addition, scientists with international expertise in the fields covered by this programme were invited to make proposals for research agreements. Several proposals were received and five (from Australia, Mexico, Peru, Sweden, and the USA) were selected for award of agreements. A technical contract was also awarded for assistance with data management and statistical analysis.

During the programme, three Research Co-ordination Meetings (RCMs) were held. The first, held in Vienna in November 1995, discussed each individual proposal in detail, modified them as necessary to ensure uniformity between projects and made recommendations for conduct of the survey. Participants were also provided with hands-on training in the use of a computer software package developed specifically for this project, named “AIDA” (Artificial Insemination Database Application).

The second RCM was held after 14 months, in February 1997 in Melbourne, Australia. The results from the surveys were reviewed, modifications to work plans were made where necessary, and the interventions to be used during the second phase of the study were discussed. The third and final RCM took place in May 1999 in Uppsala, Sweden where participants presented the results obtained during the full period of the CRP. The implications of the findings were discussed and conclusions and recommendations were made. The written manuscripts were scrutinized and any editorial improvements necessary in order to make them suitable for publication were discussed with the contract holders.

4. TECHNICAL SUPPORT

Participants in the CRP received standardized RIA kits based on a non-extraction solid-phase assay method using ^{125}I-labelled progesterone, together with appropriate standards and the assay protocol. The laboratories were also supported by an external quality assurance service from the IAEA Laboratories, Seibersdorf, Austria.

A uniform set of data recording sheets for field use, together with diskettes containing the computer database AIDA, were provided to participants at the inception of the programme. Thereafter, an additional set of procedures for statistical analysis of data, termed “GAIDA” (Guide to AI Data Analysis) was developed under a technical contract and provided to participants.

During the programme regular advise and assistance were provided to contract holders through correspondence by agreement holders and the IAEA technical officer. Where possible, on-site assistance was also provided through visits of Technical Co-operation experts and the technical officer.

5. CONCLUSIONS AND RECOMMENDATIONS

The overall conclusions and recommendations emanating from this CRP as discussed and adopted at the final RCM are given below. Specific conclusions and recommendations relating to each country participating in this CRP are given in the individual papers in this publication.

5.1. Conclusions

5.1.1. The standardized methodology and uniform approach to data recording and analysis used in this CRP have resulted in the generation of a unique international data set on the current status of artificial insemination (AI) in cattle in 14 developing countries of Asia and Latin America.

5.1.2. Measurement of progesterone by radioimmunoassay (RIA) in milk samples collected at specific times in relation to AI, combined with the use of the computer database AIDA (Artificial Insemination Database Application), has proved to be a powerful tool for calculating reproductive indices and identifying factors which affect them. The Guide to AI Data Analysis (GAIDA) system was a useful adjunct to facilitate data analysis.

5.1.3. The methodology and approach have provided a better understanding of the complex factors influencing AI programmes and, in many countries, have resulted in the first reliable assessment of the success rate of AI and the efficiency of reproductive management by small-scale dairy farmers.

5.1.4. The CRP has been a good learning experience for the participants. It has provided an opportunity to work directly with AI personnel and farmers, strengthened capability for project planning, organization and management, lead to interchange of experiences at international level and exposure to the range of problems existing in different countries, and shifted the research emphasis of participants to a more problem solving approach.

5.1.5. The results, based on over 11 000 services in 7 990 cows on 1 735 farms, have permitted a clear understanding of the major constraints and factors contributing to inefficiency of AI services. They have highlighted the need for closer monitoring of field results by AI service providers and for better education of AI technicians and farmers.

5.1.6. The conception rate to first service ranged from 15% to 62% in the study areas of the 14 countries, with an overall mean of 41%. The overall mean intervals from calving to first service and to conception were 122 and 138 days, respectively.

5.1.7. The main causes of low fertility were heat detection failure, inseminations at inappropriate time, poor semen quality, embryo mortality, seasonal influences and factors related to management on individual farms.

5.1.8. Overall, 17.3% of cows were inseminated at an inappropriate time (range 2–55% among countries). Of this, 7% (1.5–18%) were during the luteal phase or pregnancy and 10% (1–48%) were during anoestrus.

5.1.9. Of the services performed at an appropriate time, 24.7% did not result in a pregnancy as diagnosed by progesterone measurement at 22–24 days (due to non-fertilization or early embryonic death). A high proportion of these cows were not submitted for further services until rectal palpation 2–3 months later, highlighting the failure of farmers to detect subsequent returns to oestrus.

5.1.10. Of the animals diagnosed as possibly pregnant by progesterone assay, 12% were found to be non-pregnant at rectal palpation (due to late embryo mortality or persistence of luteal function).

5.1.11. The efficiency of non-pregnancy diagnosis based on progesterone assay at 22–24 days after AI was greater than 95%.

5.1.12. Interventions aimed at improving fertility were undertaken by several contract holders. These included improved nutrition, management of suckling by calves, restricting breeding to favourable seasons, education of AI technicians, synchronization and/or induction of oestrus using hormonal treatment and conduct of field fertility clinics. Adoption of some of these practices by AI services and farmers resulted in beneficial impact on reproductive performance.

5.1.13. The CRP has clearly demonstrated the value of accurately identifying the management problems as a basis for implementing interventions. It has already assisted in improving the performance of AI technicians and has been instrumental in initiating programmes aimed at improving the dairy industry in some countries.

5.1.14. The results clearly demonstrate the potential value of the progesterone RIA in providing diagnostic and related services to farmers in developing countries. Of the participants, 92% confirmed the feasibility of establishing a non-pregnancy diagnosis service, provided that some financial support and assay reagents were available during the initial phase to demonstrate cost effectiveness.

5.2. Recommendations

5.2.1. The future sustainability of the methodology used in this CRP will rely heavily on the continuous availability of cost effective reagents required for progesterone assay in developing countries. This should be ensured through appropriate regional strategies for the production and distribution of essential reagents.

5.2.2. The AIDA database has great potential for application in national AI programmes and farmer-oriented services. Its further development and customization for specific needs should be supported.

5.2.3. The survey methodology developed under this CRP should be used for monitoring and improving AI services in other areas of participating countries and also extended to other countries.

5.2.4. There is a need to focus on aspects related to male reproduction in AI services and to adopt standard procedures for identifying inefficiencies in the production, handling, transport, evaluation and utilization of semen.

5.2.5. Based on the findings of the current CRP, participants should continue to test and introduce appropriate interventions for further improvements in reproductive efficiency.

5.2.6. The links established between scientists, AI services, farming communities and extension systems must be further strengthened and developed. This should include regular monitoring, analysis and utilization of data collected in AI programmes, regular communication and interchange of information between researchers, extension staff and farmers.

5.2.7. It is strongly recommended that routine services to dairy farmers, including diagnosis of non-pregnancy and infertility based on progesterone RIA, be established.

COUNTRY REPORTS
AND
REVIEWS

CONSTRAINTS LIMITING THE EFFICIENCY OF ARTIFICIAL INSEMINATION OF CATTLE IN BANGLADESH

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Abstract

CONSTRAINTS LIMITING THE EFFICIENCY OF ARTIFICIAL INSEMINATION OF CATTLE IN BANGLADESH.

The aim of the present study was to identify the factors that influence postpartum intervals to first detected luteal activity, first service and to conception, and the conception rates of cows in the artificial insemination (AI) programme in Bangladesh. A baseline survey (investigations 1, 2 and 3) was made on 444 milking cows of various breeds presented for the first postpartum insemination by 413 farmers living at 182 villages/regions in Mymensingh District to 6 AI centres and sub-centres. Each cow was then examined three times after each AI until she stopped returning to oestrus. Sixty to 120 days after the last AI, the cows were examined per rectum to confirm the pregnancy. Milk progesterone data on Day 0 and Day 21–24 contributed to a clear diagnosis with respect to pregnancy in 82.5% cows indicating a possible use of this progesterone assay schedule for pregnancy diagnosis in AI programmes. The intervals to first service and to conception varied from 31 to 427 days (median = 184; n = 444) and 40 to 426 days (median = 184; n = 232) respectively, and conception rate from 32–58% (average 46.2%; n = 444). Prolongation of weaning age of calves resulted in long intervals to first service and to conception ($P < 0.001$); weaning age varied from 6 to 19 months (median = 10). Cows with body condition score (BCS; 1–5 scale) of 3 or more and cows calved during July to September had shorter intervals to first service and conception than those with BCS less than 3 and those calved during March. The conception rate was influenced by cattle rearing systems (intensive vs. extensive), purpose of rearing cows (dairy vs. dairy + draught), BCS and milk production ($P < 0.05$). The degrees of vulvar swelling, nature of genital discharge, tonicity of uterus, and interval between oestrus and AI had significant effects on the conception rate. Bulls classified as good and poor on the basis of semen evaluation data differed with respect to the conception rate in AI ($P < 0.001$); this indicates a way of discriminating to some extend between bulls likely to have higher or lower fertility. In Investigation 4, milk progesterone was monitored two times in a month with a 10–day interval in 88 cows. The samples were taken between 10 days after calving and the first detected oestrus followed by two more samples 10 days apart. The proportion of cows accurately detected in oestrus was 30%. Another 30% were stated to be in oestrus when they were not (false positive) and 40% were not detected when they were in oestrus (false negative). The intervals between calving and oestrus, and luteal activity were 40–362 (median = 120, n = 82) and 34–398 (median = 111, n = 64) days, respectively. The BCS at calving and at the initiation of luteal activity influenced the interval between calving and luteal activity ($P < 0.05$). Cows suckled twice daily initiated luteal activity earlier than their counterparts suckled several times daily ($P < 0.05$). Investigation 5 demonstrated a reduction in sperm motility ($P < 0.05$) due to dilution of fresh semen, and chilling, freezing, storing and transportation of frozen semen. The prolonged postpartum interval between calving and conception and low conception rate are the major constraints limiting the success of AI for cattle development in Bangladesh. The nutritional condition of the cow at calving and thereafter, weaning age of calves, frequency of suckling, cattle rearing system, accuracy of heat detection, interval between oestrus and AI, the oestrus signs and semen quality are the important determinants of the interval to conception and conception rate.

1. INTRODUCTION

Many farms in Bangladesh are so small that only one cow can be kept. Cows are tethered in a stable or on available grazing land. They are used for draught work as well as milk production and weaning is not controlled. These management practices promote the occurrence of postpartum anoestrus and limit behavioural manifestations of oestrus [1, 2, 3]. Most cows are *Bos indicus* which show weak oestrus signs for a shorter duration than *Bos taurus* cows [4, 5, 6]. Detection of oestrus and of the return to oestrus after unsuccessful artificial insemination (AI) is clearly difficult under those conditions and inefficiencies have been documented [7].

Traditionally pregnancy diagnosis is not carried out as part of the AI programmes. Veterinary services are not always available. There is therefore a need to introduce other methods to establish the status of cows with respect to cyclicity and pregnancy in association with AI programmes.

The results of AI in Bangladesh are poor with a conception rate of 37% after inseminating with chilled semen compared with 61% after natural services [8]. Yet AI has the potential to improve productivity of cows on small farms [9] if high reproductive efficiency can be achieved.

The aims of the present work were (a) to use the radioimmunoassay of progesterone in milk as a diagnostic tool to survey some aspects of reproduction in Bangladeshi cows and (b) to define the major factors limiting reproductive efficiency in smallholder farms using AI in Bangladesh.

2. MATERIALS AND METHODS

2.1. Baseline survey

2.1.1. Description of project area, AI activities, farms and cows

The investigation was carried out on 444 milking cows of various breeds presented for first postpartum AI. Most were *Bos indicus* and others were crosses of *Bos indicus* with Holstein-Friesian and a mixture of native breeds. The cows belonged to 413 farmers living at 182 villages/regions in Mymensingh District - one of the 64 administrative units in Bangladesh. The field work took place between August 1995 and June 1997. The farmers' land totalled between 0 and 40 (median = 0.6) hectares with 1–70 (median = 2.0) breedable cows.

The farmers presented their cow after 5 to 32 (median = 17) h from oestrus detection, walking 0 to 17 (median = 2.0) km, for insemination by any of the 7 inseminators; the 0 km indicates the situation where inseminations were done in the farms. The cows were being milked 1 to 2 (median = 1.0) times per day with their calves at foot. The cows were 4 to 18 (median = 7.4) years old, and their parity ranged from 1 to 12 (median = 2), body weight measured from 103 to 480 (median = 196) kg, body condition scored from 1 to 5 (median = 3) and milk production varied from 0.3 to 16.0 (median = 2) L per day. Controlled weaning was not practised, therefore, the time of spontaneous weaning was recorded. The AI technicians received 2.5 to 12 (median = 12) months formal training and had 14 to 36 (median = 16.9) years of practical experience with AI in cows. They do between 50 to 250 (median = 175) inseminations per month. Semen used belonged to 146 batches with sperm motility 65 to 75% and 29 to 70% before and after processing, respectively, from 26 bulls representing 7 breeds. Frozen semen in 0.25 mL French straws was used for 254 inseminations and 248 inseminations were made with chilled semen. In the cases of chilled semen, individual cow doses were either 1 mL (n = 195) or 2 mL (n = 53).

2.1.2. Investigation 1. Use of radioimmunoassay of progesterone in milk to survey some reproductive characteristics of cows in an AI programme

To analyse progesterone concentration, milk samples were collected in vials containing sodium azide tablets (8 mg; Merk, Darmstadt, Germany) for preservation. Milking cows presented for first postpartum insemination were included in this study. The Day 0 (day of insemination) milk samples were collected by the AI technician immediately after AI. The research personnel picked-up the samples within two days after collection. The Day 9–13 and Day 21–24 milk samples were collected by research personnel directly from the cow at a farm visit. The milk samples were centrifuged, skimmed milk was separated and stored at –20°C until analysed. Progesterone concentration in milk was determined by using solid phase radioimmunoassay (RIA) kits supplied by the FAO/IAEA Vienna. The intra-assay co-efficient of variance (CV) with internal quality control (IQC) samples varied from 8.0 to 15.6% (10 assays, each with 10 replicates) and the inter-assays CVs were 16.6% and 18.4% for beginning and end IQC samples, respectively (number of assays = 10).

The data on milk progesterone concentrations at Day 0, Day 9–13 and Day 21–24 were compared with the results of per rectum pregnancy diagnosis. Progesterone data based on two samples (Day 0 and Day 9–13) were used to examine the efficiency of oestrus detection. Milk progesterone concentration on the day of AI was used to determine the proportions of AI done in the luteal phase of the cow.

2.1.3. Investigation 2. Reproductive efficiency of cows in an AI programme

The interval from calving to first service, calving to conception and the first service conception rate were used as indices of reproductive efficiency. Four hundred and forty four milking cows presented for first postpartum AI were included in this study.

After each AI, the technician filled in the prescribed form to record information on interval from oestrus to AI, oestrous behaviour reported by the farmers, degrees of vulvar swelling and uterine tone, and the characteristics of genital discharge. The information about farms and cows was recorded between Day 9 and Day 13 at a farm visit. The age of the cow was determined by dental examination and the parities were confirmed by questioning the farmers. The body weight of the cow was estimated by using a standardised tape (Swedish Association for Livestock Breeding and Production, Eskilstuna, Sweden). The nutritional condition of the cow was scored (1–5) following modification of the methods described by Nicholson and Butterworth [10]. Between Day 60 and Day 120 after the last recorded AI, all cows were examined per rectum for confirmation of pregnancy; a milk sample was always collected immediately before rectal palpation if the cow was not dry at that time.

2.1.4. Investigation 3. Evaluation of semen and classification of bulls

Three insemination doses from different batches of individual bulls were examined for sperm motility, concentration and proportion of normal spermatozoa with respect to the acrosome, midpiece and tail [11, 12]. To grade the bulls as good or poor, criteria were set based on semen evaluation data. For chilled semen, if the insemination dose of a bull contained ≥50% sperm motility, $\geq 7.5 \times 10^6$ total motile spermatozoa, ≥70% normal spermatozoa with regard to acrosome, midpiece and tail, and ≥80% spermatozoa with normal head morphology then the bull was regarded as good. For frozen semen, the corresponding criteria were ≥30% motility, $\geq 7.5 \times 10^6$ total motile spermatozoa per insemination dose, ≥65% normal spermatozoa with regard to acrosome, midpiece and tail, and ≥80% spermatozoa with normal head morphology. If semen characteristics from a bull did not meet one or more of these criteria he was classified as poor. The values set in the criteria were the mean of data on 3 semen samples of individual bulls minus 1 standard deviation. The classification of bulls was completed before analysing fertility data.

2.2. Investigation 4: Accuracy of oestrus detection studied by using radioimmunoassay of progesterone in milk

In the area and AI programmes described in the section *2.1.1.*, the field work for this investigation was carried out during July 1997 and December 1998. Eighty-eight cows in 58 farms were registered within 1 week after calving and relevant information with regard to the farm and cattle was recorded. For individual cows, information was collected on age, breed, parity, last calving date, body weight and body condition score at calving, occurrence of oestrus, and the occurrence of any post parturient disorders. Cows that required major assistance during parturition and/or that were diagnosed with periparturient disorders like retained placenta, puerperal metritis, postpartum haemorrage, prolapse of the genital tract and milk fever were not included in the investigation. We requested the farmers to report the date and signs of oestrus but there was no discussion between the project personnel and farmers on the signs of oestrus and their relevance to conception.

The research personnel collected milk samples from individual cows twice a month at a 10-day interval between 10 days postpartum and the first report of oestrus by the farmer. Two more samples were collected at 10 days interval, after the occurrence of oestrus. During milk sampling, the research personnel scored the cow for body condition, measured her for body weight, asked the farmer about the occurrence of oestrus and examined the cow for the presence of any dry or fresh discharge adhering to the perineum. The milk samples were processed as in the case of Investigation 1.

2.3. Investigation 5. Evaluation of frozen semen from production to insemination

Five crossbred bulls were examined for breeding soundness according to the methods described elsewhere and they were regarded as clinically normal [13].

Semen from the bulls was sampled immediately after collection, initial dilution, cooling down to +4°C, and after storage at –196°C for 1 day, 7 days and 3 months in the Central Cattle Breeding Station (CCBS), Savar, Dhaka. The same batches of semen were transported to the District AI Centre, Mymensingh, and then samples were collected after 7 days, 3 months and immediately before insemination. From the District AI Centre semen was transported to the Sub Centre, Fulbaria and sampled there within 7 days after transportation and immediately before insemination. The experiment was repeated 3 times. The semen samples were examined for sperm motility and proportions of normal spermatozoa with respect to acrosome, midpiece and tail.

2.4. Analysis of data

The data for base line survey (Investigations 1, 2 and 3) were entered in a database application (artificial insemination data application; AIDA), processed in Excel worksheets and analyses were made using linear regression or a general linear model [14]. To determine the factors affecting the intervals from calving to first service and to conception, the following regression model was used:

$$INT = a + b1x1i + b2x2i + b3x3i + b4x4i + b5x5i + b6x6i + b7x7i + b8x8i + b9x9i + b10x10i + b11x11i + ei$$

where:
INT = Logs of intervals from calving to first service and to conception (days)
a = constant
x1i = total land (hectare) held by the farmers
x2i = number of breedable cows per farm (counts)
x3i = distance between the AI centres and the farms (km)
x4i = age of calves at weaning (months)
x5i = age of the cow (years)
x6i = parity of the cows (counts)
x7i = month of calving (January = 1; December = 12)
x8i = month of doing AI (January = 1; December = 12)
x9i = body weight (BW) of the cow at AI (kg)
x10i = body condition scores (BCS) of the cow at AI (1–5 scale)
x11i = milk produced by the cow at AI (kg)
ei = error term

To determine the effects of grouped and categorical variables on the intervals from calving to first service or to conception a general linear model was used:

$$INT = \mu + b1x1 + b2x2 + b3x3 + b4x4 + b5x5 + b6x6 + b7x7 + b8x8 + b9x9 + b10x10 + b11x11 + e$$

where:
INT= Logs of intervals from calving to first service and to conception (days)
μ = general mean
x1 = cattle rearing systems (extensive vs. intensive)
x2 = purpose of rearing cattle (dairy vs. dairy + draught)
x3 = frequency of suckling (once or twice daily vs. several times a day)
x4 = feeding system (concentrate added vs. no concentrate added)
x5 = breed of cows (crossbred Friesian, crossbred Sahiwal, local)
x6 = month of calving (January = 1; December = 12)
x7 = month of doing AI (January = 1; December = 12)
x8 = BCS of the cow at AI (Groups 1 = BCS 1.0–2.0, 2 = BCS 2.5–3.0, 3 = BCS 3.5–5.0)
e = error term

Chi-square test was used to determine the effect on the conception rate of the following factors:

(1) Purpose of rearing cattle: Dairy vs. dairy + draught
(2) Frequency of suckling: Once or twice daily vs. several times a day
(3) Breed of cows: Crossbred Friesian, crossbred Sahiwal, local
(4) BCS: Groups 1 = BCS 1.0–2.0, 2 = BCS 2.5–3.0, 3 = BCS 3.5–5.0
(5) Daily milk production (kg): Groups 1 = ≤1 kg, 2 = >1–2 kg, 3 = >2–4 kg, 4 = >4–16 kg
(6) Cattle rearing system: intensive vs. extensive
(7) Oestrus-to-AI interval: 1–18 h vs. 19–32 h
(8) Signs of oestrus: Standing to be mounted or mounting others, bellowing, genital discharge or restlessness
(9) Degrees of vulvar swelling: Marked vs. slight or imperceptible
(10) Nature of genital discharge: Clear mucus, turbid, none or purulent
(11) Tonicity of the uterus at AI: Marked vs. slight or imperceptible
(12) Breed of bulls: Friesian, Friesian x local, Sahiwal x others
(13) Grades of bulls: Good vs. poor
(14) Sources of semen: Imported vs. locally produced
(15) Types of semen: Frozen vs. chilled
(16) Total spermatozoa per cow dose: ≤15 million, 16–20 million, 21–25 million, 26–30 million
(17) AI technicians: n = 7

In Investigation 4, the following model was used to analyse the data:

INT= μ + b1x1 + b2x2 + b3x3 + b4x4 + b5x5 + b6x6 + b7x7 + b8x8 + b9x9 + e
where:
INT= log of interval between calving and the first detected luteal activity (days)
μ = general mean
x1 = cattle rearing system (extensive vs. intensive)
x2 = purpose of rearing cows (dairy vs. dairy + draught)
x3 = feeding system (concentrate fed vs. no concentrate fed)
x4 = breed of cows (crossbred Friesian, crossbred Sahiwal, local)
x5 = BW at calving (Groups 1 = ≤200 kg, 2 = 201–250 kg, 3 = 251–300 kg, 4 = ≥301 kg)
x6 = BCS at calving (Groups 1 = BCS 1.0–2.0, 2 = BCS 2.5, 3 = BCS 3.0, 4 = BCS 3.5–5.0)
x7 = BW at the first detected luteal activity (Groups 1 = ≤200 kg, 2 = 201–250 kg, 3 = 251–300 kg, 4 = ≥301 kg)
x8 = BCS at the first detected luteal activity (Groups 1 = BCS 1.0–2.0, 2 = BCS 2.5, 3 = BCS 3.0, 4 = BCS 3.5–5.0)
x9 = frequency of suckling (once or twice daily vs. several times a day)
e = error term

The relationship between the postpartum period to initial luteal activity and the estimated first rise in concentration of milk progesterone was tested by using Pearson correlation. The estimated rise concentration refers to the mean of the first detected progesterone rise (≥1 nmol/L) and the progesterone concentrations in samples immediately before and after the first rise. ANOVA was used to test the effect of the accuracy of oestrus detection on the postpartum interval to oestrus (false positive cases were deleted) and on the estimated progesterone concentration at the first peak. Unless otherwise indicated, the data are presented as median and range owing to high individual variations. Only the factors that tended (P = 0.10) to or significantly influenced the dependent variables are presented in the Figures and Tables.

In Investigation 5, repeated measures analysis of variance with different contrast was used to analyse data on sperm motility and proportion of normal spermatozoa (with regard to the arosome, midpiece and tail) obtained from fresh ejaculates, and from semen after dilution, chilling and equilibration, freezing, storage at CCBS for 7 days and 3 months, transportation to the District AI Centre and storing there for 3 months, thawing and preparation for insemination at the District AI centre, transportation to an AI Sub Centre, and after thawing and preparation for insemination at the Sub Centre [14]. In general, the data were log-transformed to near normality except the proportion values which were transformed by arcsine transformation.

3. RESULTS

3.1. Investigation 1. Use of radioimmunoassay (RIA) of progesterone in milk to survey some reproductive characteristics of cows in an AI programme

The interpretation of progesterone data based on three samples (Day 0, Day 9–13 and Day 21–24) with respect to pregnancy results is shown in the Table I. Milk progesterone data gave a clear interpretation on 82.5% cows (n = 360) about their pregnancy status when comparison was made with the data of per rectum pregnancy diagnosis at Day 60–120. None of the 75 cows with a progesterone profile of low (<1.0 nmol/L), high (≥3 nmol/L) and low on Day 0, Day 9–13 and Day 21–24, respectively, were found pregnant at rectal palpation. Twenty-seven cows (7.5%) had a progesterone profile of low high and high on Day 0, Day 9–13 and Day 21–24, respectively, but were not pregnant at rectal examination. Two inseminations were made in pregnant cows. All cows confirmed pregnant at rectal palpation had a high level of progesterone in milk at the day of pregnancy diagnosis. Eighty one percent of cows (n = 478) had a progesterone profile of low and high on Day 0 and Day 9–13, respectively, indicating AI done not at luteal phase or during ovarian acyclicity (Table II). Fifty-nine cows (12.3%) received AI at an incorrect time as evident from a deviant progesterone profile. Twenty- seven of 506 (5.3%) cows were inseminated when (Day 0) they had high to intermediate level of progesterone in milk.

TABLE I. MILK PROGESTERONE DATA FROM THREE SAMPLES AND INTERPRETATION IN RELATION TO MANUAL PREGNANCY DIAGNOSIS

Day 0 (day of AI)	Day 9–13	Day 21–24	Number of cases (%)	Rectal palpation results; interpretation
[1]Low	[2]High	High	202 (56.1)	Pregnant
Low	[3]Intermediate	High	7 (1.9)	Pregnant; RIA problem, biological variations
Low	High	Low	75 (20.8)	Non-pregnant; fertilisation failure, early embryonic death, post AI anoestrus
Low	Intermediate	Low	7 (1.9)	Non-pregnant; fertilisation failure, short luteal phase, RIA problem, biological variation
Intermediate /high	Low/inter-mediate/high	Low	6 (1.7)	Non-pregnant; AI at incorrect time, post AI anoestrus
Clear interpretation			297 (82.5)	
Low	High	High	27 (7.5)	Non-pregnant; late embryonic death (>16 days) luteal cyst, persistent corpus luteum (CL)
High	High	High	2 (0.6)	Pregnant; AI on pregnant animal
Low	Intermediate	High	4 (1.1)	Non-pregnant; RIA problem, biological variation, late embryonic death, persistent CL
Low	High	Intermediate	20 (5.6)	Non-pregnant; fertilisation failure, late embryonic death RIA problem, biological variation
Low	Low	Intermediate	2 (0.6)	Non-pregnant; AI in anoestrous cow, RIA problem
Intermediate/ High	Low/inter-mediate/high	Intermediate /high	8 (2.2)	Non-pregnant; AI at incorrect time, luteal cyst, persistent CL
Total number of observations			360	

[1]Low = <1.0 nmol/L, [2]High = ≥3.0 nmol/L, [3]Intermediate = between 1.0 and 3.0 nmol/L

TABLE II. MILK PROGESTERONE DATA ON THE DAY OF SERVICE AND ON DAY 9–13 WITH RESPECT TO THE ACCURACY OF OESTRUS DETECTION

Day 0 (days of AI)	Day 9–13	Number of cases (%)	Interpretations
[1]Low	[2]High	387 (81.0)	Progesterone concentration within negative range on Day 0 and within positive range on Day 9–13 indicates an ovulatory cycle-accurate oestrus detection.
Low	Low	47 (9.8)	Progesterone concentration within negative range on both days indicates anoestrus, anovulation, or short luteal phase-inaccurate oestrus detection.
High	High	6 (1.3)	Progesterone concentration within positive range on both days indicates AI in pregnant animals or in animals with luteal cyst-inaccurate oestrus detection.
High	Low	6 (1.3)	Progesterone concentration within positive range on Day 0 and within negative range on Day 9–13 indicates that AI was performed during luteal phase-inaccurate oestrus detection.
Total occurrence		478	
Total inaccurate oestrus detection		59 (12.3)	

[1]Low = <1.0 nmol/L, [2]High = ≥3.0 nmol/L, [3]Intermediate = between 1.0 and 3.0 nmol/L. Thirty-two (6.7%) services were made in cows with an intermediate level of milk progesterone on Day 0, Day 9–13 or on both occasions.

3.2. Investigation 2. Reproductive efficiency of cows in an AI programme

The intervals from calving to first service and to conception, and the mean first service conception rate were 31–427 (median = 184; n = 444) and 40–426 (median = 184; n = 232) days and 46.2% (n = 444), respectively.

The results for the intervals between calving and first service, and conception rate are shown in Figures 1 to 4. The effect of age of cows on the intervals from calving to first service and to conception was not significant (P >0.10). The effect of body condition at AI was significant on the postpartum intervals to the first service (P <0.001) and to conception. (P <0.05), and on the conception rate (P <0.001). Cows with BCS 3.5 or more took shorter intervals to receive first postpartum service and to conceive than those with BCS 3 or less (Figure 1A and 1B; P <0.01). The conception rates of cows with BCS 1.0–2.0, 2.5–3.0 and 3.5–5.0 were 36%, 46% and 64%, respectively. The crossbred Sahiwal cows had a shorter calving to first AI interval than the crossbred Friesian and the pure local cows (Figure 1C; P <0.05). The crossbred Sahiwal cows had a shorter postpartum interval to conception than the crossbred Friesian and local cows (Figure 1D); in both cases, the differences between crossbred Sahiwal and crossbred Friesian were significant (P <0.05). The differences between breeds in conception rate was not significant (P = 17; 54% in crossbred Friesian, 48% in crossbred Sahiwal and 43% in local cows).

The postpartum intervals to first service and to conception increased with increasing age of the calves at weaning (Figure 2A and 2B; P <0.001). The relationship between daily milk yield at first AI and the intervals to the first postpartum AI was not significant (Figure 2C and 2D, P >0.10). The effect of milk production on the conception rate was significant (P <0.05). Cows producing ≤1.0, >1–2, >2–4 and >4–16 kg milk daily had conception rates of 36%, 45%, 50% and 58%, respectively. The dairy cows took shorter times after calving to receive first service (median = 174, 31–427 days; n = 315) and to achieve conception (median = 181, 40–405 days; n = 175) than the dairy +draught cows; the median interval from calving to first AI was 214 (53–426; n = 129) days, and to conception

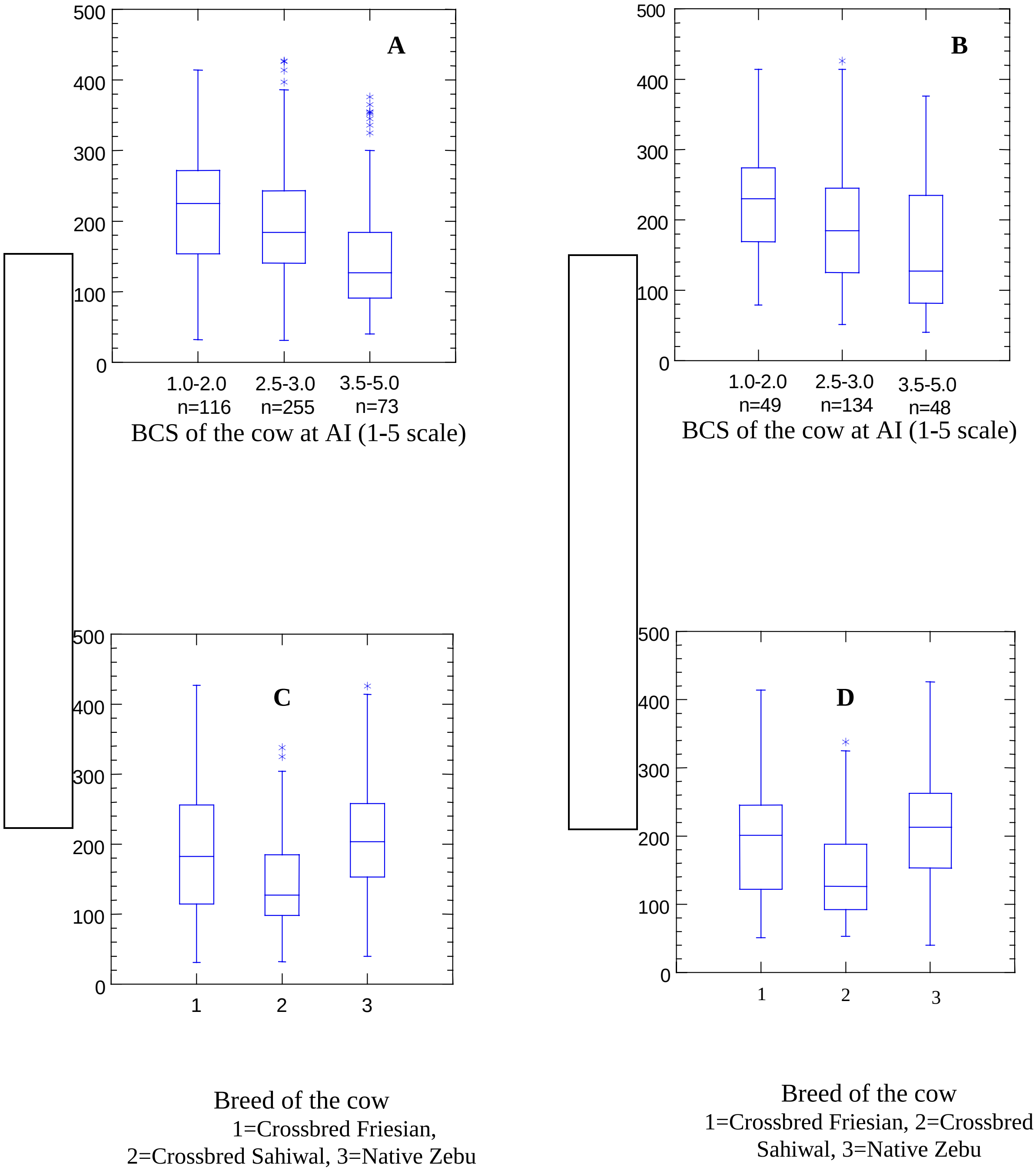

FIG. 1 (A-D). Effect of body condition score and breed of cows on the intervals from calving to first AI and to conception

was 233 (53–426; n = 57) days (P <0.05). Similarly, the conception rate was higher (P <0.05) in dairy cows (50%) than that in dairy + draught cows (38%).

Cows managed intensively tended to conceive at a higher (P = 0.05) rate (53%; n = 156) than those reared extensively (43%; n = 288). However, the effects of rearing system on the intervals between calving and first service (median = 167, 31–427 vs. 212, 40–426; intensive vs. extensive), and conception (median = 172, 53–376; n = 79 vs. 203, 40–426; n = 153; intensive vs. extensive)

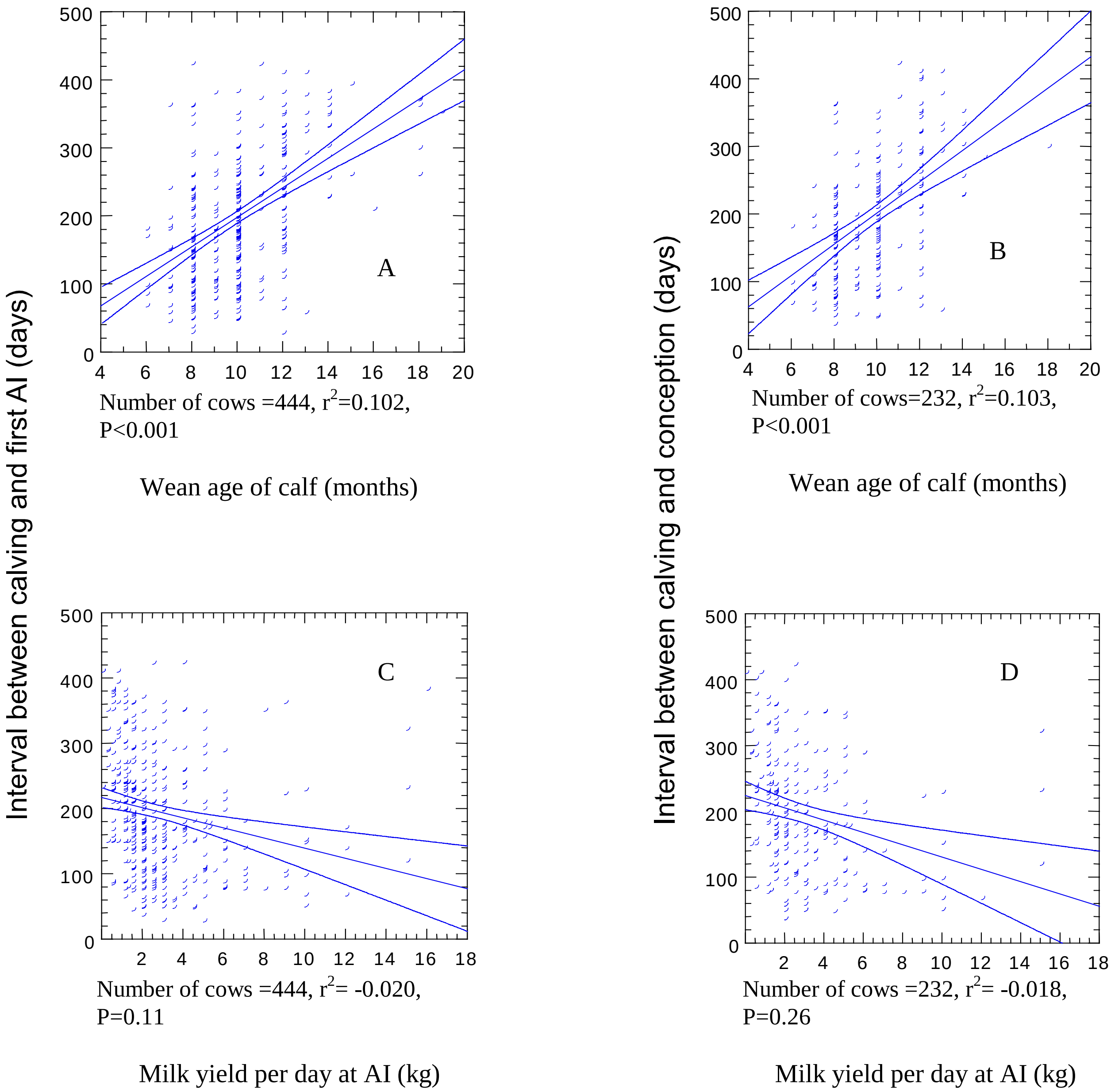

FIG. 2(A-D). Relationship of wean age of calves and milk yield of cows with the post partum intervals to first AI and to conception.

were not significant (P >0.5). Cows suckled twice or less tended (P = 0.10) to have shorter intervals between calving and first service (median = 168, 52–427; n = 162), and conception (median = 167, 53–426; n = 95) than those suckled several times daily; median postpartum interval to first service was 203 (31–414; n = 282) and to conception was 202 (40–414; n = 137). The conception rate of once or twice suckled cows (53%) tended to be higher (P = 0.09) than those suckled several times daily (42%).

Cows served between 5 and 18 h after being detected in oestrus conceived at a higher rate (49%; n = 366) than those served between 19 and 32 h (32%; n = 78; P <0.01). Cows detected in oestrus on the basis of mounting activity, bellowing and genital discharge or restlessness conceived at a rate of 49% (n = 245), 45% (n = 161) and 32% (n = 38), respectively; the differences between groups were not significant (P = 11). Conception rate was higher (P <0.05) in cows with marked vulvar swelling (53%; n = 241) than those with slight or imperceptible swelling (38%; n = 203). Cows with clear, turbid and purulent or no genital discharge conceived at a rate of 51% (n = 335), 43% (n = 35) and 27% (n = 74), respectively; the differences between genital discharge-groups were significant (P <0.05). Cows with marked uterine tone at AI conceived at a higher (P <0.001) rate (54%; n = 284) than those with slight or imperceptible uterine tone (33%; n = 160). Depending on AI technicians, the conception rate varied from 31.3% to 54%; however, the differences between technicians were not significant (P = 0.06).

Cows calved during July to September took a shorter time to receive first postpartum AI than those calved in March (Figure 3A; P <0.05). The month of calving as main effect on the interval between calving and conception was significant (P <0.05); however, after Bonferroni Adjustment, the differences in post partum interval to conception between months were not significant (Figure 3B; P >0.10). Similarly, the month of AI as main effect appeared significant on the intervals from calving to first service (P <0.01) and to conception (P <0.05) but after Bonferroni adjustment, the differences between months were not significant (Figure 3C, D; P >0.05).

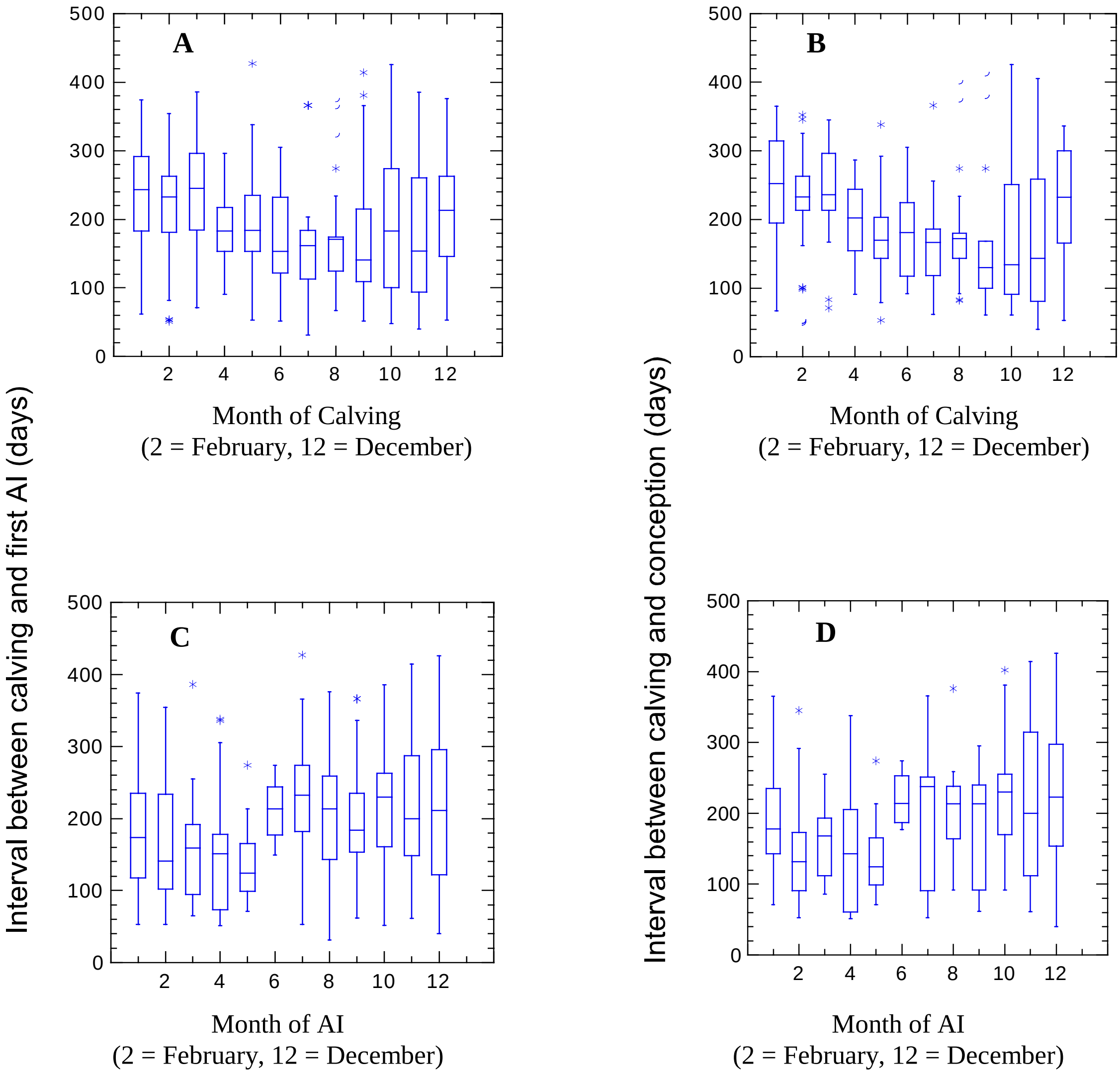

FIG. 3. (A-D) Effect of month of calving and month of AI on the post partum intervals to first AI and to conception

3.3. Investigation 3. Evaluation of semen and classification of bulls

Breed of bulls influenced the first service conception rate ($P < 0.001$). Friesian bulls had higher ($P < 0.01$) fertility (56.5%, n = 184), than did Friesian x local (43.9%, n = 114) and Sahiwal x others (34.9%, n = 146). The fertility of good bulls (52.6%, n = 266) was higher ($P < 0.001$) than that of poor bulls (35.8%, n = 165). Thirteen inseminations were made with semen of bulls with incomplete information and therefore remained unclassified. The use of frozen semen resulted in higher ($P < 0.001$) conception rate (54.9%, n = 224) than did the chilled semen (37.3%, n = 220). The imported frozen semen yielded higher ($P < 0.001$) conception rate (57.5%, n = 174) than did the locally produced semen (38.9%, n = 270), irrespective of frozen or chilled-preserved. The days of chilling and sperm motility of preserved semen did not influence the conception rate ($P > 0.50$). The use of 1 and 2 days chilled semen resulted in 37.2% (n = 129) and 37.9% (n = 91) conception, respectively. The sperm motility of <50%, 50–60% and >60–70% resulted in conception rates of 49.2% (n = 63), 50.0% (n = 86) and 44.4% (n = 295), respectively (ns). The total spermatozoa per cow dose influenced the conception rate ($P < 0.001$). A total of 15 million or less spermatozoa resulted in lower ($P < 0.05$) conception rate (27.0%, n = 100) than did 16–20 million (44.0%, n = 134), 21–25 million (58.3%, n = 108) and 26–30 million spermatozoa (54.9%, n = 102).

3.4. Investigation 4: Accuracy of oestrus detection studied by using radioimmunoassay of progesterone in milk

The cows studied were 3–15 (median = 6.0) years old and 1 to 8 (median = 3.0) parities. At calving they weighed 160–456 (median = 270) kg and scored body condition 1.5 to 3.5 (median = 2.5). The intervals after calving to the first detected oestrus and to the initiation of luteal activity were 40–362 (median = 115; n = 82) and 34–398 (median = 108, n = 64), respectively. The estimates of progesterone concentration at first rise varied from 0.5 to 9.3 (median = 2.4). Farmers missed detecting an oestrus (false negative) on 1 to 3 (median = 1.0) occasions postpartum. The proportion of cows accurately detected in oestrus was 30%. Another 30% were stated to be in oestrus when they were not (false positive) and 40% were not detected when they were in oestrus.

The intervals to the initiation of first postpartum luteal activity were examined in these cows. Those with BCS 3.5 or more at calving needed fewer days to initiate luteal activity than their counterparts having BCS 3.0 or less (Figure 4A, B; $P < 0.05$). Cows suckled once or twice daily required fewer days (median = 95, 34–398; n = 28) than the cows suckled several times (median = 127, 35–279; n = 36) ($P < 0.05$).

The median interval to first postpartum oestrus was prolonged by 33.5 days due to farmers' inability to detect the oestrous cows ($P < 0.05$). Cows missing detection of oestrus had lower milk progesterone concentration at first luteal activity (median = 1.8, 0.5–8.1; n = 34) than those detected accurately in oestrus (median = 4.2, 0.5–9.3; n = 26) ($P < 0.05$). A positive relationship appeared between the estimated progesterone concentration during the first peak and the duration of postpartum acyclicity ($r^2 = 0.284$, n = 64, $P < 0.05$). The age of the cows and their parities did not influence the initiation of luteal activity ($P > 0.50$). The effects of body weight at calving, purpose of rearing cows (dairy vs. dairy + draught) and breed of cows on the initiation of luteal activity were not significant ($P > 0.10$).

3.5. Investigation 5. Evaluation of frozen semen from production to insemination

The percentage of motile spermatozoa varied from 37.9 ± 5.8 to 65.4 ± 6.6, depending on the occasions when evaluation was made. The results are shown in Figure 5. The sperm motility dropped due to dilution ($P < 0.05$), chilling ($P < 0.05$), freezing ($P < 0.001$) and storing 3 months in the Bull Station ($P < 0.01$). Three months storage in the District AI Centre also reduced ($P < 0.05$) sperm motility. Transportation of semen from the District AI Centre to the Sub Centre caused further reduction ($P < 0.05$) in the sperm motility.

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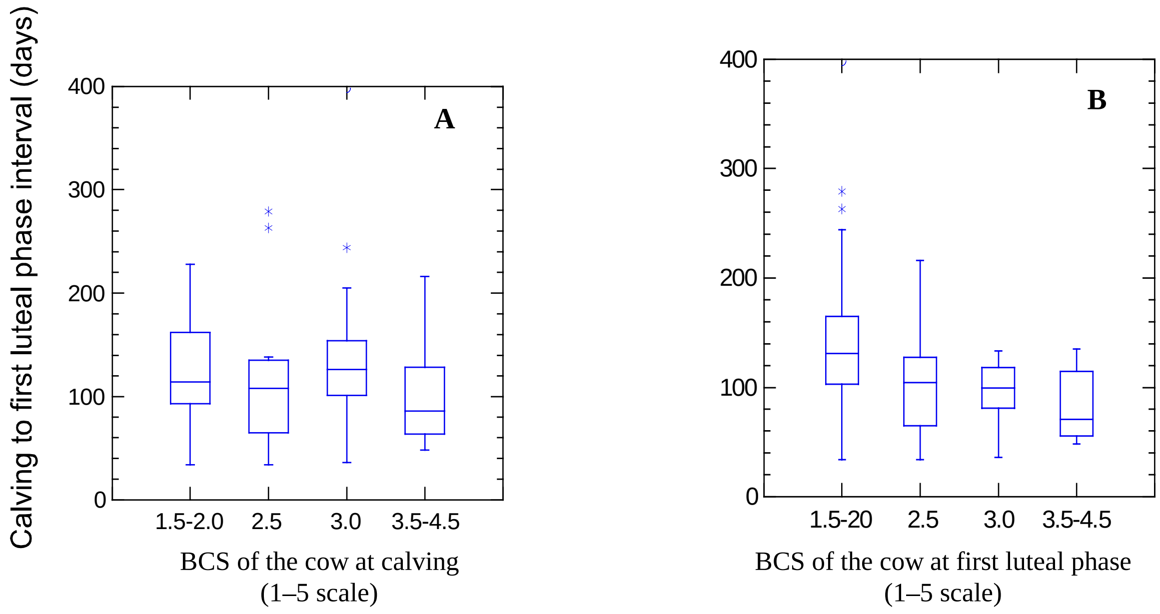

FIG. 4. (A, B) Effect of body condition at calving and at first luteal phase on the post partum interval to the initiation of luteal activity.

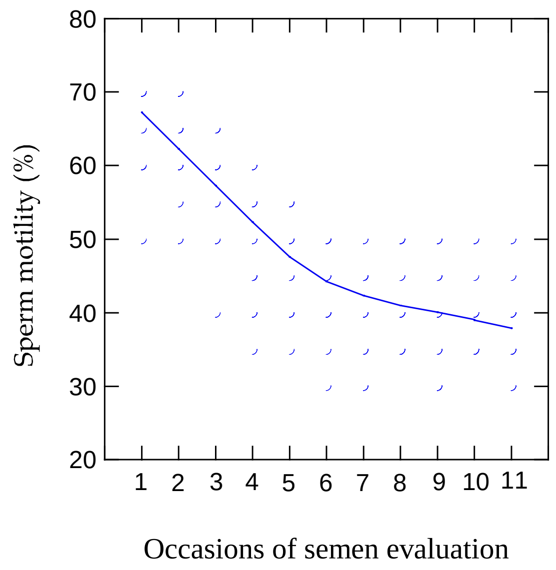

FIG. 5. Sperm motility in fresh ejaculate, and at different stages of semen freezing, storage, transportation and preparation for AI. 1 = fresh ejaculate (65 ± 7%), 2 = diluted with tris-egg yolk medium (62 ± 6%), 3 = diluted with tris-egg yolk-glycerol medium, cooled down to 4°C and equilibrated for 4 h (58 ± 8%), 4 = frozen-thawed semen (48 ± 8%), 5 = thawed after 1 week of freezing (47 ± 7%), 6 = transported from the Bull Station to the District AI Centre (44 ± 8%), 7 = prepared for AI in the District AI Centre (41 ± 6%), 8 = stored 3 months at the Bull Station (42 ± 6%), 9 = stored 3 months in the District AI Centre (40 ± 7%), 10 = transported from the District AI Centre to a Sub Centre (38 ± 8%) and 11 = prepared for AI in the Sub Centre (38 ± 6%). Note the values in the parenthesis are the mean ± SD of 15 observations.

The percentage of normal spermatozoa with respect to the acrosome, midpiece and tail were between 84.6 ± 4.8 and 90.0 ± 5.2 depending on the occasions of sampling; the percentage dropped (P <0.01) due to freezing semen (Figure 6).

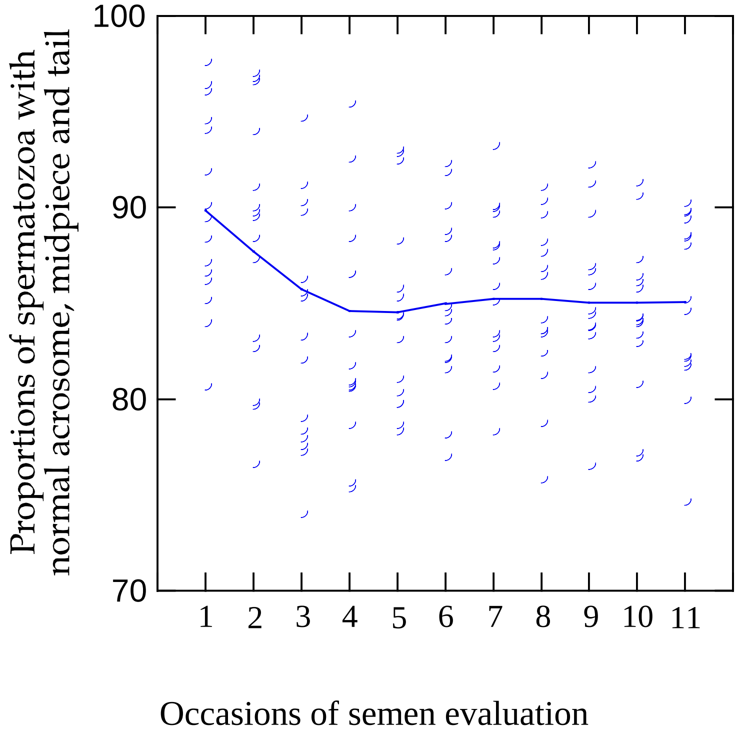

FIG. 6. The proportion of spermatozoa with normal acrosome, midpiece and tail in fresh ejaculates, and at different stages of freezing, storage, transportation and preparation for AI. 1 = fresh ejaculate (90 ± 5%), 2 = diluted with tris-egg yolk medium (89 ± 7%), 3 = diluted with tris-egg yolk-glycerol medium, cooled down to 4°C and equilibrated for 4 h (84 ± 6%), 4 = frozen-thawed semen (84 ± 7%), 5 = thawed after 1 week of freezing (85 ± 5%), 6 = transported from the Bull Station to the District AI Centre (85 ± 5%), 7 = prepared for AI in the District AI Centre (86 ± 5%), 8 = stored 3 months at the Bull Station (85 ± 5%), 9 = stored 3 months in the District AI Centre (85 ± 5%), 10 = transported from the District AI Centre to a Sub Centre (85 ± 4%) and 11 = prepared for AI in the Sub Centre (85 ± 5%). Note the values in the parenthesis are the mean ± SD of 15 observations.

4. DISCUSSION

4.1. Investigation 1. Use of radioimmunoassay of progesterone in milk to survey some reproductive characteristics of cows in an AI programme

Milk progesterone data based on three samples (Day 0, Day 9–13 and Day 21–24) helped make a clear decision in 82.5% cows about the pregnancy. If only the pregnancy diagnosis is concerned, milk progesterone concentration on the day of AI and on Day 21–24 will give the same results without compromising the accuracy. Milk progesterone concentration on Day 0 and Day 9–13 together not only identified the inaccurate oestrus detection but also gave a clear idea about the cyclical status of the animals. Progesterone concentration on Day 0 can only indicate whether or not AI was done in a cow with functional luteal tissues in the ovary. In the case where veterinary services are not available or are too expensive, milk progesterone concentrations on the day of AI and on Day 21–24 can be used to interpret pregnancy results with more than 80% accuracy; diagnosis of non-pregnancy was highly accurate.

4.2. Investigation 2. Reproductive efficiency of cows under AI programmes

The main findings were the prolonged intervals from calving to first service and to conception. Poor nutrition condition during calving and thereafter, frequent suckling of long duration because of no practice of weaning, use of cows in draught power, high proportion of inaccurate oestrus detection are, among others, important determinants of the calving to first service interval. The local cows seem to have an inherent tendency towards long interval from calving to first service; this may not always get corrected by cross breeding with Friesian cattle. The system and purpose of rearing cows, BCS at AI, milk production at AI, interval between oestrus and AI, degrees of vulvar swelling, nature of genital discharge and the tonicity of uterus at AI significantly influenced the first service conception rates.

Long interval to conception after calving not only results in fewer calves during the lifetime of a cow and hence hinders anticipated genetic gain but also reduces the use of AI in the industry. When the number of AI's per technician increases, costs per cows are reduced. Low cost is one of the important prerequisites for farmers' acceptance of AI [15].

The favourable effects of good body condition of cows on the interval to conception demonstrated here have also been shown by other workers [16, 17, 18, 19]. Good body condition of cows is only maintained if the negative energy balance due to production of milk is covered by adequate nutrition and management [16, 20, 21]. The longer intervals to first service and conception in dairy + draught cows than their dairy counterparts, evident from our results, further supports the significant role of post partum energy balance on the reproductive events. However, the effect of chronic stress on the cow caused by draught work should not be overlooked [22]. Stress prevents cows from showing characteristics behavioural and clinical signs of oestrus [23]. Well developed oestrous signs, like marked vulvar swelling, clear, stringy mucus discharge and marked tonicity of the uterus proved in this study to have favourable effects on conception rate.

The results of this study demonstrated that the cows calved during July to September took shorter time to receive the first postpartum AI and to conceive than those calved during December to March, although the differences were not always significant. The study area experiences hot and humid climate during April to September; green forages are mostly available during July to September. In contrast, the climatic conditions during December to March in Bangladesh are cold and dry. During December-March, the cultivable lands are occupied by crop under irrigation programme and the barren lands are too dry to support growth of grass for communal grazing. The interactions between climatic factors, nutritional factors and genetic factors in causing reproductive inefficiency are complex. Further work is needed on the specific effects of green forage and on alternative methods of nutritional management.

In contrast to the data on recognized dairy breeds [24], our results indicated a positive relationship between milk yield at AI and the intervals to first postpartum service and conception, although this was not statistically significant. The situation can be explained by the fact that dairy + draught cows yielding less milk proved to have longer postpartum intervals to the first service and conception. The milk production in this study has been recorded on the day of insemination. It is likely that cows with prolonged postpartum intervals due to other reasons were about to dry off and consequently being considered as low-yielding. Also it is usual that high yielding cows are paid more attention, fed well and reared under good management; all these have favourable effects on the intervals to the first service and conception. The latter situation is supported by the fact that high yielding cows in our studies conceived at a higher rate than their low producing counterparts.

It appears in that the overall intervals (median) from calving-to-first service and to conception are similar. This is because most of the cows received AI only on first post partum oestrus and then did not receive further inseminations even though they did not conceive on the first service.

4.3. Investigation 3. Evaluation of semen and gradation of bulls

The present study indicated that good and poor bulls as classified on the basis of semen evaluation data differed with respect to the conception rate in artificial insemination. The criteria to judge a bull as good or poor were set according to the motility, total number and morphological normality of the spermatozoa. The motility itself is a flagellar function of spermatozoa and in accordance with Söderquist [25], the present investigations did not find any relationship between sperm motility and fertility. Motile spermatozoa can be morphologically abnormal [26]. The proportion of morphologically abnormal spermatozoa in semen correlates negatively with fertility results [25, 26, 27, 28, 29].

Spermatozoal number per cow dose significantly influenced the conception rate in this study. This is in accord with the importance of the number of functionally normal spermatozoa as a determinant of fertility [30]. It also takes into account Söderquist's recommendation [25] that semen parameters need to be defined quantitatively rather than qualitatively to be able to predict the fertility. Using a combination of semen characters, as was done here, has also been recommended in attempts to evaluate semen to predict fertility [31, 32]. Innate fertility of the bull combined with optimal sperm numbers are also important in determining fertility in an AI programme [30]. The significant effect of bull-breed on conception rate found in the present study is further confirmation of this. The classification used here discriminated to some extend between bulls likely to have higher or lower fertility. Further work is warranted in ranking bulls in more categories under conditions where greater numbers of cows with confirmed fertility results can be used.

The data of the present study reveal that cows inseminated with frozen semen conceived at a higher rate than those inseminated with chilled semen. This is in contrast with some earlier reports where the conception rate after insemination with chilled semen was higher than that obtained after insemination with frozen semen, provided the preservation temperature remained constant [33, 34, 35, 36]. The low conception rate after insemination with chilled semen in this study could be due to difficulties in maintaining constant temperature during transportation and storage of chilled semen.

4.4. Investigation 4: Accuracy of oestrus detection studied by using radioimmunoassay of progesterone in milk

Failure to detect oestrus (false negative) and false determination of oestrus (false positive) are common problems in AI of cows in intensive farming [5, 37, 38, 39]. In accordance with our results, false negative and false positive categories of oestrus detection have been found by others to be as high as 30 to 50% [5, 37, 39] and 17 to 30% [38], respectively. The false positive oestrus detection was lower in Investigation 1 (12%) than that in Investigation 3 (30%). In Investigation 1, the cows were recorded at the AI centres. Firstly, this means, farmers made a rigid decision about the oestrus of the cow. Secondly, the registration of cows by the AI technicians in Investigation 1 raises the question as to whether they made any selection in favour of good oestrus sign to prove their good performance. Thirdly, in Investigation 4, to avoid our frequent visits, some farmers might have made intentional false report about the oestrus. Because cows in the false negative category are not inseminated and cows in the false positive category are unlikely to get pregnant in that cycle, the calving to conception interval is increased in all cases.

Cows with good body condition at calving and thereafter initiates postpartum cyclicity earlier than those in poor body condition. This indicates the importance of good nutritional management of pregnant cows to cover the negative energy balance due to growth of foetus. As shown in Investigation 2, early initiation of postpartum cyclicity will reduce the interval from calving to first service and to conception.

The adverse effect of the duration and frequency of suckling on the initiation of postpartum cyclicity as indicated by our results are in accordance with other reports [40]. Also in Investigation 2, frequent suckling tended to increase the intervals from calving to first service and to conception. Controlled suckling for a restricted period favours postpartum reproduction in cows [41]. The results of the present investigations do not clarify whether prolonged suckling lengthens the onset of postpartum ovarian activity or the suckling continues because the cows do not dry off since they are not pregnant. However, in commercial dairy farming, controlled weaning should be practiced to identify the cows with an inherent tendency to remain acyclic irrespective of suckling and nutrition management.

The low concentration of progesterone at the first postpartum rise of false negative cows raises question as to whether their oestrous cycle was regular or not. There are reports of short oestrous cycle [17, 42] and transient rises in progesterone before the onset of usual ovarian activity [17, 43, 44, 45]. However, one should interpret such data cautiously, especially when progesterone profiles are monitored in less frequently sampled milk as in the case of our investigations. None the less inadequate luteinization often results from defective follicular development due to lack of LH-receptor activity [43]. There are also claims about an organizing effect of transient progesterone rise on the ovarian-pituitary-hypothalamic axis to facilitate the re-establishment of regular oestrus cycle [43, 44].

4.5. Investigation 5. Evaluation of frozen semen from production to insemination

The sperm motility dropped significantly due to dilution of fresh semen, chilling, freezing, storing in the bull station and in the District AI Centre and transportation from the District AI Centre to Sub Centre. Similarly, the proportion of normal spermatozoa significantly reduced due to freezing of semen. As a result, the cow received semen with 38% and 40% average sperm motility in the District AI Centre and Sub Centre, respectively. This means, the cow receives 12.0 million or less motile spermatozoa, given 30 million total spermatozoa per cow dose. The importance of the number of spermatozoa per cow dose was evident in Investigation 3. The freezing protocol, and handling of frozen semen during transportation and management during storage need to be improved to ensure at least 50% sperm motility at the time of AI, given 30–35 million total spermatozoa per cow dose.

5. CONCLUSIONS

It appears that determination of progesterone concentration in milk on the day of AI and on Day 21–24 is a good means to make decision on pregnancy results by diagnosing the non-pregnant state with high accuracy. Prolonged postpartum intervals to the initiation of ovarian activity, first service and conception, and low conception rate are the major constraints limiting the development of cattle by AI in Bangladesh. The nutrition condition of the cow, duration and frequency of suckling, use of cows in draught power and accuracy of heat detection are, among others, the important determinants of the intervals to the initiation of the ovarian activity, and to the first service and conception. The classification of bulls into good and poor based on semen evaluation data discriminated to some extend between bulls likely to have higher or lower fertility. The end-users in Bangladesh receive frozen semen with 12 million or less motile spermatozoa per cow dose; this needs to be improved to achieve good fertility.

From these studies several areas emerge as important for future work. The progesterone assay and data analysis system used here should be introduced as a service to farmers to assist in reproductive management of their farms. Improving nutrition will clearly benefit the cows' reproductive efficiency. Economic studies should be carried out to formulate cost effective strategies, especially for small farmers. The monitoring of bulls and semen should be continued. There is a need to improve the production and handling of semen to achieve high quality at the point of insemination. Farmers should have better training on oestrus detection. Further training and workshops should be instituted for AI technicians to achieve a more uniform and higher fertility results.

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USE OF MILK PROGESTERONE RIA FOR THE MONITORING OF ARTIFICIAL INSEMINATION IN DAIRY COWS

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Abstract

USE OF MILK PROGESTERONE RIA FOR THE MONITORING OF ARTIFICIAL INSEMINATION IN DAIRY COWS.
Milk samples were collected on day 0, day 10–12 and day 22–24 after artificial insemination (AI) from 2349 dairy cows in 5 dairy farms. Progesterone concentration was measured by RIA. Based on the progesterone concentration in the three milk samples, the reproductive status of the cows could be identified and they were classified as pregnant (50.9%), non-fertilisation (25.8%), inactive ovary (6.1%), persistent corpus luteum (3.5%), AI at inappropriate time (during luteal phase or luteal cyst, 6.2%) and abnormal oestrous cycles (7.5%). The results and interpretation were sent back to AI technician and veterinarians in the dairy farms as soon as possible. They in turn used this information, together with their findings from rectal palpation, to arrive at a reliable diagnosis of the reproductive status in each cow and, where necessary, to adopt appropriate remedial measures in order to ensure pregnancy at subsequent service. So far, 3477 oestrus cycles have been monitored. For establishing a routine system of milk progesterone monitoring in these dairy farms, an ELISA method would be more practical.

1. INTRODUCTION

One of the important aspects for increasing the productivity in dairy husbandry is to enhance the reproductive efficiency, particularly the conception rate obtained through artificial insemination (AI). However, it is known that as the individual milk yields of cows increase, reproductive problems also increase and there is a higher incidence of reproductive diseases.

At present in most dairy farms in China, the rectal palpation method is used for diagnosing pregnancy, confirming oestrus and detection of reproductive diseases. However, this is a difficult technique, which needs experience and practice. Accuracy of rectal palpation is also limited and there is a possibility of false diagnoses. Therefore this method needs to be aided by other diagnostic methods.

Use of milk progesterone assay for monitoring of reproductive state in dairy cows has a history of more than twenty years in the world [1, 2, 3]. In the last 15 years some Chinese agricultural colleges and research institutes have conducted milk progesterone assay (RIA or EIA) for early pregnancy diagnoses, oestrus confirmation and even diagnosis of reproductive diseases. But many of these reports only deal with limited numbers of cows or provide inadequate information due to the lack of frequent and regular sampling during a reproductive cycle [4, 5, 6].

In the present research the objective was to adopt an adequate milk sampling regime, and to include sufficient numbers of cows, so that we could do detailed monitoring of the reproductive process of dairy cows, not only for early non-pregnancy diagnoses, but also to detect various abnormal reproductive situations. Our purpose is to play a bigger role for enhancing the reproductive efficiency in dairy cows in China.

2. MATERIALS AND METHODS

2.1. Survey

2.1.1. Farms

Beijing area is located in a temperate zone with four distinct seasons. The highest ambient temperature during summer is above 35°C and the lowest during winter is below –10°C. There are about 20 000 dairy cows in Beijing area, most of which are Holstein breed, distributed over 45 dairy farms. Five dairy farms were chosen for this project in 1995. One of the farms (Liangzhong - LA), is owned by the government while the other four (1A, 2A, 3A and 4A) belong to the West Suburb Milk Cow Company, which is a co-operative. The average size of farms was 400 breedable cows. The purpose is purely milk production and the feeding system is based on concentrates plus roughage with no grazing.

2.1.2. AI system and technicians

The dairy farms in Beijing area are not permitted to keep breeding bulls and all cows are bred by AI. Frozen semen, packaged in straws, is purchased mainly from Beijing Bull Station, but some semen is also imported from USA and Canada). The dairy farms have their own resident AI technicians. Each farm in the present study had a chief technician who had at least ten years of AI experience and two assistants.

2.1.3. Oestrus detection and pregnancy diagnosis

Oestrus detection was done mainly by visual heat signs (standing, mounting, mucus). Some AI technicians confirmed heat by additional rectal palpation one day before AI. Pregnancy diagnosis was performed by AI technicians through rectal palpation, generally 60 days after AI, on cows which did not return to service.

2.1.4. Milk sampling

The AI technicians collected three milk samples from each inseminated cow (day 0, day 10–12 and day 22–24 after AI) and this was repeated if a cow returned to service, until she became pregnant or were culled. collection. Milk (7–8 mL) was collected directly into sampling vials which contained a preservative ($K_2Cl_2O_7$), mixed and stored at 4°C until transferred to the laboratory. The samples were centrifuged for 15 minutes at 2000G at 4°C, the fat layer was drawn off with a glass rod, most of the skim milk transferred to storage vials using a pipette, and stored at 4°C until RIA.

Samples with inadequate records or incomplete sets of samples were discarded. Milk samples from a total of 3471 services in 2349 cows were analysed and used in the interpretation of results.

2.1.5. Data recording and analysis

Data relating to the cows and services were collected from the cow cards and breeding calendars kept in the dairy farms. The information was entered into the Artificial Insemination Database Application (AIDA, Joint FAO/IAEA Division, Vienna) and reports were generated using the application.

2.2. Interventions in cows with dysfunctional ovaries

2.2.1. Reproductive management

In the farms used for the present study the AI technicians were responsible for AI and all aspects of reproductive management including diagnosis and treatment of ovarian dysfunction and the administration of exogenous hormones. The veterinarians were responsible for all other types of health problems and disorders, including prevention and treatment.

2.2.2. Commonly used exogenous hormone treatments

Cows with reproductive disorders were treated with exogenous hormones on a routine basis according to the practice commonly adopted on these farms. Prostaglandin analogue (Cloprostenol, ICI, UK) was administered at a dosage of 400 μg to cows with persistent corpus luteum (CL) or luteal cysts and, if oestrus occurred, AI was done two days after treatment. Gonadotrophin Releasing Hormone (GnRH, LRH-A3) was administered at a dosage of 20 μg to cows with inactive ovaries or follicular cysts and AI was done simultaneously. Oestradiol benzoate or a combination of steroid hormones (oestradiol, progesterone and testosterone) was administered to anoestrous cows with detectable follicles and AI was done when oestrus was observed.

2.2.3. Milk sampling

On two farms, the cows which were treated with exogenous hormone (101 and 130 cows respectively) were monitored using progesterone measurement in milk. Milk samples were collected before hormone injection, followed by the three sample regime described above (section 2.1.4). If the cows did not show oestrus after hormone treatment, two milk samples were collected on days 10–12 and 22–24 after treatment to determine the causes of anoestrus.

The data from these problem breeder cows were recorded and studied in relation to milk progesterone profiles in order to determine the underlying causes and to evaluate the effects of hormone treatment.

2.4. Radioimmunoassay

Progesterone was measured in milk samples using a direct (non-extraction) solid phase radioimmunoassay with ^{125}I labelled progesterone as tracer (FAO/IAEA, Vienna). The progesterone standards were prepared in skim milk and ranged from 0 to 40 nmol/L. All tubes were set up in duplicate. After adding standards, QC, samples and ^{125}I progesterone, all tubes were incubated for four hours at room temperature. The supernatant was then decanted and the tubes were counted in a manual gamma counter (FT-646A), with data processing software which automatically constructed the standard curve and calculated progesterone concentration in the samples.

The inter-assay coefficients of variation (CV) for low and high IQC samples were 9.7% (n = 11) and 10.3% (n = 14) respectively; the intra-assay CVs for these samples were 3.5% (n = 4) and 4.3% (n = 4) respectively. Progesterone concentrations were categorised as Low (< 1 nmol/L), Intermediate (1–3 nmol/L) and High (> 3 nmol/L).

3. RESULTS

3.1. Survey

3.1.1. Conception rate

The conception rate (CR) at first service was 44.0% and the overall CR was 42.8%. In the individual dairy farms (LA, 1A, 2A, 3A, 4A), the CR at first service was 39.6, 48.5, 47.5, 48.6 and 35.3% respectively. The overall CR in the five farms was 40.8 47.7, 43.5, 49.7 and 36.8% respectively. The average interval from calving to first service was 111.7 days and the interval from calving to conception was 132.6 days. The CR of cows decreased with increasing age (Table I).

TABLE I. CONCEPTION RATE (CR) OF COWS OF DIFFERENT AGES

Age (years)	No. of cows	No. conceiving	CR (%)
2	296	132	44.5
3	169	80	47.3
4	167	82	49.1
5	157	61	38.8
6	118	48	40.6
7	75	29	38.6
8	29	8	27.5
9	19	6	31.5
>10	14	5	35.7

3.1.2. Progesterone

Progesterone concentration was measured in 3656 samples of milk collected on the day of AI (day 0). Of these, 3324 samples (91%) had low values (<1 nmol/L), which indicate that these AI were in cows without active corpus luteum. There were 181 samples (5%) with high values (>3 nmol/L), which indicate that AI was done during luteal phase or pregnancy. The remaining samples (4%) had intermediate values (1–3 nmol/L). The results from analysis of progesterone in three samples of milk and the interpretations are given in Table II.

TABLE II. PROGESTERONE CONCENTRATION IN THREE SAMPLES OF MILK AND RESULTS OF PREGNANCY DIAGNOSIS (PD) IN THE INSEMINATED COWS

Day 0	Day 10–12	Day 22–24	PD	N° of cows	%	Interpretation
Low	High	High	Pos	1495	43.0	Conceived
Low	High	Low	Neg	892	25.8	Non-fertilisation, early embryonic mortality
Low	High	High	Neg	275	7.9	Late embryonic mortality
Low	Low	Low	Neg	212	6.1	Inactive ovary, anoestrous
High	High	High	Pos/Neg	122	3.5	AI on pregnant cows, luteal cyst, persistent CL,
*	*	*	?	476	13.7	*Intermediate values
Total				3477	100.0	

Feedback was provided to the AI technicians and veterinarians on the dairy farms as soon as possible (within 40 days after AI), on the results of progesterone assay and our interpretation. They then adopted therapeutic treatment without delay to ensure that cows got pregnant as soon as possible. Those not responding to treatment were culled.

3.2. Interventions in cows with dysfunctional ovaries

The results of progesterone assay on samples of milk collected from cows which were treated with various hormones by the AI technicians were used to assess whether the treatment was appropriate or not, and whether it was effective in overcoming the problem. The criteria for judgement were as follows.

Prostaglandin (Cloprostenol) treatment was considered appropriate if the progesterone value was high at the time, indicating existence of a CL, and inappropriate if progesterone was low. The treatment was considered effective if progesterone values decreased and the cow showed heat, whereas it was considered ineffective if progesterone values did not decrease or the cow did not show heat.

GnRH treatment was considered appropriate if progesterone value was low at that time, indicating the absence of a CL, and inappropriate if the progesterone value was high, indicating that the cow was in the luteal phase or had a luteal cyst or persistent CL. Treatment was considered effective if progesterone values rose and the cow developed a palpable CL. It was considered ineffective if progesterone did not rise and the cow did not develop a palpable CL.

The results are presented in Table III and show that 21.7% (33/152) of prostaglandin treatments and 11.5% (3/26) of GnRH treatments were inappropriate.

TABLE III. ASSESSMENT OF THE APPROPRIATENESS AND EFFECTIVENESS OF EXOGENOUS HORMONE TREATMENTS BASED ON PROGESTERONE MEASUREMENT

Farm	Hormone Used	N º cows treated	Assessment of treatment and outcome		
			Appropriate and effective (%)	Appropriate but ineffective (%)	Inappropriate (%)
LA	Prostaglandin	80	32 (40.0)	41 (51.2)	7 (8.8)
	GnRH	21	13 (61.9)	5 (23.8)	3 (14.3)
L1-L4	Prostaglandin	72	27 (37.5)	19 (26.4)	26 (36.1)
	GnRH	5	1 (20.0)	4 (80.0)	0
	Oestradiol	30	13 (43.3)	17 (56.7)	0
	Combination of steroids*	23	8 (34.8)	15 (65.2)	0

*Oestradiol, progesterone and testosterone

4. DISCUSSION

4.1. Advantages of monitoring AI by milk progesterone assay

The reproductive management level of dairy farms in Beijing area is higher compared with those in other areas of China. Mean annual milk yield was above 7000 kg and the average CR at first AI was above 50%. The incidence of infertility (5%) and abortion (7%) were low. However, the AI technicians are not always able to diagnose the status of ovarian activity correctly and they sometime administer inappropriate therapies, resulting in loss of money and time. If progesterone measurement can be introduced as a routine method into dairy farms, AI technician and veterinarians would hold a powerful tool for dairy reproductive management and the reproductive efficiency and economic benefit would be further improved. The present survey has provided very valuable information to improve reproductive management.

Of the cows with low, high and high progesterone in the three successive samples, nearly 16% returned to oestrus within two months after insemination, or were diagnosed as non-pregnant during rectal palpation. This represents 8% of the total number of AI monitored, and is due mainly to late embryo mortality during the period 25–60 days after insemination and to a lesser extent to abnormal oestrous cycles (prolonged luteal phase). This finding is similar to those in previous reports of 8% by Bulman and Lamming [7] and 12% by Yang and Guo [8]. In fact, milk progesterone monitoring is the only way for confirming embryo mortality, which is useful for identifying the causes such as management, immunity, genetics, etc. We identified and classified embryo mortality depending on progesterone patterns and the intervals from AI to recurrence of oestrus. The results indicated that most of the embryo mortality (65.5%) occurred before 50 days after AI, and the average interval from calving to conception in cows with embryo mortality was 165.7 days, which was much longer than the intervals in cows which conceived to the first AI (average 115.3 days) [9].

The cows with low, high and high progesterone in the three samples did not conceive or had early embryo mortality. In such cases the farmer must observe carefully for the next oestrus or, if there is an abnormality in the genital system, have appropriate treatment provided so that subsequent insemination will be successful. Progesterone monitoring will allow the farmer to identify non-pregnant cows earlier and avoid delays in getting them pregnant.

Presence of low progesterone in all three samples signifies that these cows had inactive ovaries, with a few possibly having follicular cysts. This information is valuable to adopt appropriate therapeutic treatment depending on the individual case, using GnRH or pregnant mare serum gonadotrophin (PMSG) to stimulate inactive ovaries, and human chorionic goadotrophin (HCG) to treat follicular cysts.

High progesterone in all three samples indicates that the cows had persistent CL, luteal cysts, or were pregnant at the time of AI. Before attempting any treatment, it is important to correctly diagnose the status of each cow by rectal palpation and to exclude those that are pregnant. For the non-pregnant cows, prostaglandin can be given to destroy the CL or luteal cysts.

The cows, which had high progesterone concentration at the time of AI were incorrectly diagnosed in oestrus. Alerting farmers to this fact helps to improve their oestrous detection system and to rule out cows in false oestrus. Such cows accounted for 5% of total inseminations, which is similar to previous reports of 4.8% [2] and 7.8% [10]. Although AI at inappropriate time due to incorrect oestrus detection is one factor that influences reproductive efficiency, other reproductive problems such as non-fertilisation and embryo mortality are of greater importance in the farms studied.

Intermediate progesterone values (1–3 nmol/L) were obtained for about 14% of the samples. This could be due to abnormal oestrus cycles, sample mishandling (milk degeneration) or inaccuracies in the assay. Other clinical data can help to interpret such results, but care is also needed to avoid errors in sampling and assay.

4.2. Possibility of establishing milk progesterone assay system in dairy farms

Monitoring of milk progesterone on AI cows, together with rectal palpation, provides a more comprehensive way to determine physiological states and to diagnose reproductive disease. It is a very useful tool for AI technicians and veterinarians working on dairy farms, which can enhance reproductive efficiency, increase CR and reduce the calving interval. However, RIA equipment and progesterone RIA kits are expensive. Also, strict regulations govern the use of radioactive materials. On the other hand, the Enzyme-Linked Immunosorbent Assay (ELISA) method of measuring progesterone can be established more cheaply. Equipment costs involve about RMB Yuan 6000 to 8000 for a plate reader and the cost of measuring each sample is RMB Yuan 3 using kits. This system would be more suitable for use on dairy farms in China.

4.3. Monitoring exogenous hormone therapy in problem cows

On normal dairy practice, to monitor all breedable cows as we have done during this study is impossible due to limitations in budget and labour. However, it would be acceptable to monitor the cows which are subjected to exogenous hormone treatment. During our survey and implementation stages, we discovered that the exogenous hormone injections were often incorrectly used in some dairy farms. For instance, some cows with persistent CL were injected with oestradiol or GnRH, and some cows with inactive ovary were injected with prostaglandin. The causes of inappropriate hormone therapy were mainly misdiagnosis of different types of ovarian dysfunction by rectal palpation (e.g. misjudgement of follicular cyst from luteal cyst). If farm technicians could take a milk sample before hormone treatment at the time of rectal palpation and determine progesterone content immediately by ELISA, most misdiagnoses would be avoided. Thus the proportion of inappropriate and unnecessary hormone treatments would be reduced greatly, resulting in economic benefits.

5. CONCLUSION

Due to the limitations of relying on visual observation and rectal palpation for determining reproductive status, monitoring milk progesterone can help to improve reproductive efficiency and economic benefits. AI technicians could lay emphasis on monitoring the cows which are subjected to exogenous hormone treatment. This would reduce the costs and labour involved in the procedure.

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THE USE OF PROGESTERONE RIA TO INCREASE EFFICIENCY AND QUALITY OF ARTIFICIAL INSEMINATION SERVICES OF BEEF CATTLE IN SOUTH SULAWESI, INDONESIA

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Abstract

THE USE OF PROGESTERONE RIA TO INCREASE EFFICIENCY AND QUALITY OF ARTIFICIAL INSEMINATION SERVICES FOR BEEF CATTLE IN SOUTH SULAWESI, INDONESIA.

The technique of artificial insemination (AI) has been used in Indonesia for several years. The fertility rate resulting from this technique, however, is still lower than that for natural mating. Therefore, two studies were conducted to evaluate the factors that might cause lower efficiency of AI in beef cattle. The first was a survey to evaluate reproductive performance and factors that affect the inefficiency of AI. A total of 652 beef cows inseminated on one large and several small farms were used. Data for farm, cow, inseminator and each insemination were recorded. Serum samples were taken at the day of insemination (day 0), and days 10–12 and 20–22 after AI, and at time of manual pregnancy diagnosis. The second study was conducted to evaluate whether the induction of first postpartum oestrus in suckling cows could be done by hormonal treatments. Two groups of suckling Brahman cows were used, with and without treatment using Controlled Intra-vaginal Drug Release devices (CIDR). CIDR were inserted into the vagina, oestradiol benzoate (OB) was injected, the CIDR were removed after 12 days and two AIs were performed at 24 and 72 hr later. The studies were conducted during dry and wet seasons. Mean overall conception rate (CR) for the large and small farms were 23 and 38%, respectively. Season of the year, housing system, type of heat, time of AI, and characteristics of AI technicians (level of education and non-AI work) affected the CR. Oestrus behaviour was detected in 95 and 100% of cows respectively for the first and the second period. However, the proportions of animals showing ovulatory oestrus during the first and second periods were 22 and 48% ($P < 0.01$), and pregnancy rates were 12 and 24%, respectively. These findings show that reproductive rate of cows served by AI in this area is low and that factors related to the cow, farm management and AI technician affect this problem. CIDR treatment can induce oestrus behaviour in suckling cows, but only few of these cows have normal ovulation followed by pregnancy. RIA for progesterone measurement is a technique that can increase the efficiency of AI.

1. INTRODUCTION

The province of South Sulawesi is one of the main resources of beef cattle in Indonesia. In 1994, the population of beef cattle in this area decreased drastically to nearly 50% of what was officially recorded in 1992 [1]. The reason for this problem is not yet fully understood. Low reproductive rate in combination with other factors may be one of the reasons. It has been reported that the calving interval of beef cattle in this area is between 2–3 years. An artificial insemination (AI) program has been applied by the government for several years in order to solve the problem of the decreasing cattle population. This program, however, has not yet been successful enough to increase the reproductive rate.

The AI technique has been widely applied for the rapid improvements of genetic and reproductive efficiency both in the developed and developing countries. In Indonesia this AI was introduced for the first time in the early 1950's and in South Sulawesi an AI program started in 1975 [2]. The fertility rate of inseminated cows, however, is still low. The reason for this has not yet been clarified. A high reproductive performance depends on the fertility of both the cow and the bull, management of the farm and the skill and ability of the AI technicians.

Progesterone is secreted by the corpus luteum (CL) throughout the luteal phase of the oestrus cycle and during pregnancy in cattle [3]. Therefore, profiles of this hormone can be used to pinpoint the reproductive status and, when measured in blood samples collected at appropriate times in relation to AI, the results can be used to evaluate if the inseminated animals are pregnant or not [4, 5]. Furthermore, incidence of factors such as insemination at the wrong time and ovarian dysfunction can also be evaluated.

Suckling and low feed intake are considered to cause delay of ovarian function in postpartum cows and result in long calving intervals [5, 6]. In suckling animals, the induction of first oestrus might be done by injection of gonadotrophin and steroid hormones [5, 7]. Also, progesterone released by intravaginal devices have been successfully applied for oestrus induction in cows [7].

This study was conducted to find out: (a) factors that might influence the efficiency of AI service of beef cattle in South Sulawesi, Indonesia; and (b) whether the onset of first postpartum oestrus could be induced by hormonal treatment.

2. MATERIALS AND METHODS

Two studies were conducted: the first was a survey to evaluate reproductive performance of inseminated beef cattle and factors that might influence the efficiency of the AI service; the second was an experiment to examine whether the onset of the first postpartum oestrus could be induced by hormonal treatments.

2.1. Study one

2.1.1. Location

This study was conducted in the South Sulawesi province from July 1995–July 1997. This is a tropical humid area with an average yearly rainfall of 2843 mm and ambient temperature ranging between 22 and 32°C. There are two seasons, dry (April to September) and wet (October to March). Of the 20 districts that are using AI, three (Bantaeng, Sidrap and Maros) were randomly chosen for the study.

2.1.2. Farms and Animals

The survey included 261 small farms and one large farm (PT Berdikari United Livestock, Sidrap, about 200 km north of Makassar). On the large farm animals are kept for beef production. The animals in the small farms are mainly used for draught and to a lesser extent for beef production. The large farm has about 1500 breeding cows and the small farms have between 1 and 10 cows. On the large farm 266 Brahman cows were selected for insemination and were subjected to oestrus synchronization using a prostaglandin analogue (Prosolvin). Inseminations were performed by two technicians.

On the small farms 386 cows were inseminated at spontaneous oestrus by 15 technicians. All animals were local Bali cattle and their crosses with exotic breeds (Limousin, Hereford, Brahman, Simmental and Ongole cross breeds).

All semen used for insemination were frozen in 0.25 mL straws and were produced at the National AI Center. Before shipment from the AI Center the straws contained at least 10 million live sperm. Breeds of bulls were Bali, Limousine, Simmental and Brahman. The semen was thawed in water at ambient temperature before insemination.

2.1.3. Procedures

During the survey, information on the farm, cow, inseminator and semen batch were recorded in accordance with the data entry sheets for the AIDA database [8]. For progesterone analysis, blood samples were taken by the technician on the day of insemination (day 0), day 10–12, day 20–22 and at pregnancy diagnosis. Pregnancy diagnoses were performed by rectal palpation at least 2 months after AI.

Blood samples were centrifuged soon after collection (less than 4 h) and the serum was stored at –20°C until progesterone assay. Progesterone concentration in the serum samples was measured by radioimmunoassay (RIA) technique [9]. Intra- and inter-assay coefficients of variation were 5.6 and

10.8%, respectively. The profiles of progesterone values were constructed for individual cows and were used to evaluate ovarian activity and outcome of insemination.

2.2. Study two

This study was conducted at the large farm of study one (PT Berdikari). The period of study was from May 1998 to January 1999 (end of dry season to middle of rainy season) and suckling Brahman cross cows that were 70–90 days postpartum were used.

The study was divided into 2 periods: (P1) May–July 1998, the end of dry season; and (P2) September 1998–January 1999, middle of rainy season. One group of cows (P1; n = 50, P2; n = 21) received progesterone treatment through Controlled Intra-vaginal Drug Release (CIDR) devices (Inter-Ag). The CIDR was inserted into the vagina for 13 days. Oestradiol benzoate (OB) was injected 24 hours after withdrawal of the CIDR and AI was performed 48–72 hr after the removal of the CIDR. Another group of cows (P1, n = 50; P2, n = 20) received no treatment, served as a control group and were kept separately from the treated animals. Blood samples were taken from all animals once during CIDR treatment, on the day of AI (day 0), on days 10, 20, 30 and 40 after AI and at pregnancy diagnosis on day 60 after AI. Progesterone concentration in serum samples were measured by RIA as described above.

Body condition score (BCS, judged on a scale from 1 to 5) and intensity of oestrus behaviour (scale from 1 to 3) were recorded. Progesterone concentrations in serum were used to calculate number of animals showing ovarian activity and conceiving after treatment.

2.3. Data analysis

Data were entered and tabulated using the AIDA program and were analysed for factors affecting reproductive performances in the SYSTAT programme. Frequency tables were used for data tabulation separately for the large farm and the small farms. Chi-square was used to test hypotheses.

3. RESULTS

3. 1. Study one

3.1.1. Reproductive performance

The reproductive performance of cows in the two groups of farm is shown in Table I.

TABLE I. REPRODUCTIVE PERFORMANCE OF COWS ON THE LARGE FARM AND SMALL FARMS

Parameter	Large farm	Small Farms
Interval (days) from calving to:		
- First service	269.66 ± 97.0	150.80 ± 90.0
- Conception after first service	259.40 ± 93.6	145.10 ± 70.9
Services per conception	4.4	2.6
Conception rate (%):		
- At first service	22.8	26.3
- Overall	23.0	38.3

3.1.2. Factors affecting conception rate (CR)

(a) Effects of cow on CR

There were two factors related to cows that affected the CR in small farms: season of AI service and type of heat (natural or after oestrous synchronisation). The CR was higher during the rainy season (October – January compared to the rest of the year (P <0.01). In the small farms 8.7% of the cows were inseminated after oestrous synchronisation with a CR of 22.7%. The rest were inseminated after natural heat with a CR of 39.8% (P <0.05). In the large farm there were no cow factors that influenced the CR.

(b) Effect of farm management on CR

In the small farms CR was influenced by housing system and time of AI. Animals kept in corrals or paddocks had higher CR (45.7%) than those kept in other housing systems (P <0.01). Inseminations done in the morning (AM) resulted in a higher CR compared to those done in the afternoon (44.6% vs. 28.0%, P <0.01). In the large farm, where all animals were synchronised to oestrus, inseminations in the afternoon resulted in a higher CR (31.5%) than those in the morning (20.8%).

(c) Effect of AI technician on CR

Among the AI technicians, the level of education and non-AI work affected CR. Technicians who had graduated from high school had higher CR than those who had only primary school education (47 vs. 21.6%) (P <0.01) and those who were working full time on AI had higher CR than those working part time (46.7 vs. 22.4%, P <0.01).

(d) Effect of bull on CR

There was no significant influence of bulls on CR in any of the farm types.

3.1.3. Interpretation of progesterone data

The number of inseminations for which all three sera samples were available for progesterone analysis was 174 in the large farm and 135 in the small farms. The interpretation of the data are presented in Table II.

TABLE II. RESULTS FROM ANALYSIS OF SERUM PROGESTERONE FROM THREE SAMPLES AND MANUAL PREGNANCY DIAGNOSIS

Progesterone (nmol/L) on days			Manual pregnancy diagnosis	Number (Frequency %)		Interpretation
0 (AI)	10–12	22–24		Large Farm	Small Farms	
Low	High	High	Positive	45 (34.1)	41 (35.7)	Pregnant
Low	High	Low	Negative	25 (18.9)	26 (22.6)	Non-fertilization, early embryonic mortality, post AI anoestrus
Low	High	High	Negative	0	0	Late embryonic mortality, luteal cyst, persistent CL
High	High	High	Positive	2 (1.5)	0	AI on pregnant animals
*	*	*	Positive/ Negative	42 (24.1)	20 (14.8)	Intermediate level of progesterone (1–3 nmol/L)
Total occurrence				174	135	

The number of inseminations for which two serum samples were available for progesterone analysis was 208 in the large farm and 223 in the small farms. The proportion of inseminations performed at the correct time (progesterone low on day 0 and high on day 10–12) was 55.5% in the large farm and 58.6% in the small farms. Anoestrus, anovulation or a short luteal phase was detected in 26.0% of the inseminations done in the large farm and in 30.8% of those in the small farms, while the inseminations performed during the luteal phase were 9.8 and 8.1% respectively. Samples that had intermediate concentrations (1–3 nmol/L) where so no conclusions could be drawn accounted for 16.8% in the large farm and 11.2% in the small farms.

The number of inseminations for which only the day 0 serum sample was available was 262 for the large farm and 323 for the small farms. The proportions of inseminations performed when progesterone was low were 86% in the large farm and 88.4% in the small farms, while those done at an incorrect time (progesterone high) were 18.6% and 11.6% respectively.

3.2. Study two

3.2.1. Body condition score (BCS)

BCS of the animals was lower during P1 than during P2 (P <0.01). In P1, nearly 50% of the animals had a BCS of 2 (low), while in P2 more than 50% had a BCS of 3 (medium), and more than 20% had a BCS of 4 (good).

3.2.2. Oestrus behaviour

All animals treated with CIDR+OB showed oestrous signs (swollen vagina, vaginal mucous discharge and mounting each other) after treatment in both periods, whereas none of the control animals did so. The intensity of oestrous behaviour is given in Table III.

TABLE III. OESTROUS SIGNS AFTER TREATMENT WITH CONTROLLED INTRA-VAGINAL DRUG RELEASING DEVICES AND OESTRADIOL BENZOATE DURING THE TWO PERIODS

Oestrous Signs	Period 1 (%)	Period 2 (%)
Not apparent	10	0
Apparent	34	57
Very Apparent	56	43

3.2.3.Ovarian activity and pregnancy rate

The frequency of animals that expressed oestrus, showed ovarian activity and became pregnant after treatment with CIDR+OB are presented in Fig. 1. The percentage of animals with ovarian activity was significantly higher in P2 than in P1 (48% vs. 22%, P <0.01). The pregnancy rate (number of pregnant cows/number of cows with ovarian activity) was not significantly different between periods (54 vs. 50%). All animals that showed ovarian activity, except one cow in period two, returned to anoestrus (with progesterone levels continuing to be low). In the control groups, only 1 out of 50 animals (2%) in P1 and 3 out of 21 (14%) in P2 showed ovarian activity.

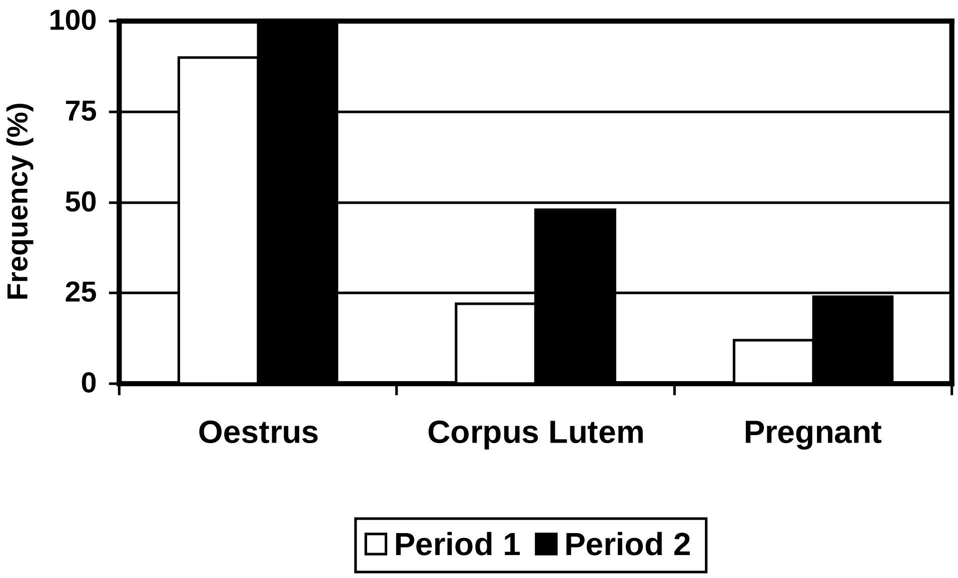

FIG. 1. The combined frequency of animals that expressed oestrus, showed ovarian activity (Corpus Luteum) and became pregnant after treatment with CIDR+OB in periods 1 and 2.

4. DISCUSSION

Long intervals from calving to first AI service and to conception, as well as low CR, were noted in beef cows under the conditions prevailing in South Sulawesi, Indonesia. Factors related to the cow, management of the farm and inseminator were correlated with the low reproductive efficiency. Treatment with progesterone and oestrogen could induce the onset of early postpartum oestrus in suckling cows. However, only a limited number of animals showed ovarian activity after treatment and few animals became pregnant.

In order to maximise productive life, a cow should be bred within 80 to 90 days after calving [5, 7]. The mean interval from calving to first service recorded in this study was much longer (199.1 days). This may have been caused by management factors such as the failure of farmers to detect oestrus and to breed animals during the oestrus period. Since most of the cows in this area are raised extensively with the purpose mainly for beef and draught, factors such as suckling, nutrition and season could be responsible for the prolonged anoestrous period postpartum [5]. Other factors, such as management of the farms and housing will also suppress the expression of oestrus in cows [7].

Mean interval from calving to conception found in this study was 162.7 days. This will give a calving interval of around 440 days, which is much longer than the optimum interval of 12 to 13 months [5, 7]. One way to reduce this interval would be to perform pregnancy diagnoses earlier in the postpartum period. Since progesterone is secreted by the corpus luteum throughout pregnancy, the analysis of this hormone in blood or in milk has been widely used in pregnancy diagnosis [6]. In a review by Peters and Ball [5] the accuracy of this technique for positive pregnancy diagnosis was 80–85%, and for negative diagnosis almost 100%. Therefore, the cows which do not conceive to AI can be detected at an early stage and the farmer can take necessary action to detect heat and mate the cow.

The CR recorded in this study (32.3%) was low when compared with those reported in developed countries using AI [6, 10]. However, it was similar to that previously reported under natural mating in this area of Indonesia [1]. Wattiaux [7] has stated that reproductive efficiency is a multifaceted subject, and may be affected by at least four factors: fertility of cow, fertility of bull, efficiency of heat detection and efficiency of insemination. In the present study, factors considered included those related to the cow (body condition, intensity of oestrus), farm's condition (location, management) and background of inseminator (level of education, job intensity).

This study has confirmed that the low CR was the result of several factors such as season and time of AI, types of heat, housing system, educational level of technician and whether the technician is performing AI only or other work as well. Higher CR when cows were inseminated during the wet season could be due to the greater availability of feed and the decrease in heat stress. This is also confirmed by the finding that CR of cows exposed to sunlight was lower than that of cows kept under shade. Thus a change of management where shade is provided during the dry season should improve fertility. Cows inseminated in the morning had higher CR than those inseminated in the afternoon. This could be due to a high percentage of cows coming in to heat during the night, and the timing of AI being better when done in the morning. However, it would be possible to maximise the heat detection efficiency by increasing the frequency of observations to 2–3 times per day.

It should be noted that the above effects were detected mainly in the small farms. The higher fertility seen in the large farm could be attributed to better management and the fact that they had their own AI technicians who were well trained.

There has been much interest in methods to induce oestrus and ovulation. These include use of exogenous gonadotrophins, stimulation of gonadotrophin secretion and indirect stimulation of GnRH release [4]. Stimulation of gonadotrophin secretion can be done through initial suppression using progestagen treatment followed by its withdrawal. One such treatment is through the use of CIDRs. In the present study CIDRs successfully induced oestrus behaviour in suckling beef cows. However, some of these oestrous animals failed to ovulate and continue ovarian activity. Anovulatory oestrus has been reported to occur even without any noticeable abnormalities in the preovulatory hormonal events [11] and the reasons for its occurrence after progesterone treatment are not known. Since this incidence was mostly detected during the dry period when the BCS was low, the effectiveness of this treatment might be affected by nutrition and related factors. Similar result has been also reported in Brahman heifers during the hot season in a tropical climate [12].

5. CONCLUSIONS

Based on the above results, it is concluded that:

- Beef cows inseminated in the study area have low reproductive efficiency, manifested as long interval from calving to first AI and low CR. Factors related to the cow (season, BCS and type of oestrus), farm management (purpose of rearing cattle, housing system and timing of AI), and AI technician (level of education and type of work) influenced the results.
- Progesterone treatment uaing CIDR + oestradiol benzoate effectively induced estrus behaviour in suckling cows, but a high proportion of them failed to ovulate. The incidence of anovulation was higher during the dry season.
- Progesterone profiles determined using the RIA technique had a high correlation with ovarian activity and outcome of AI, and can be used to increase the efficiency of AI.

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IMPROVEMENT OF CATTLE PRODUCTION IN MYANMAR THROUGH THE USE OF PROGESTERONE RIA TO INCREASE EFFICIENCY AND QUALITY OF ARTIFICIAL INSEMINATION SERVICES

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Abstract

IMPROVEMENT OF CATTLE PRODUCTION IN MYANMAR THROUGH THE USE OF PROGESTERONE RIA TO INCREASE EFFICIENCY AND QUALITY OF ARTIFICIAL INSEMINATION SERVICES.

A survey of Artificial Insemination (AI) status in Myanmar was carried out in the Mandalay region. Most farms are smallholdings with 1–12 breedable cattle per farm. During the survey a total 435 first inseminations carried out by 5 AI technicians were recorded. The conception rate (CR) at first service was 60.7% and the overall CR was 63.3%. Interval from calving to first service was 103.6 ± 40.0 days. Progesterone measurement on the samples collected on the day of AI (day 0) showed that 6.3% of the services were done when progesterone was high (>3nmol/L), indicating that the cows could not have been in oestrus. Most of the farmers detected oestrus based on signs such as mucus discharge, bellowing and mounting. After the preliminary survey a study was conducted to test of two intervention measures: to reduce the interval from calving to first service by nutritional supplementation with urea molasses multinutrient blocks (UMMB); and to reduce the number of AI done during the luteal phase. In this study 245 first AI were recorded. Interval from calving to first oestrus was 95.8 ± 24.8 days. Incidence of AI at luteal phase declined to 4%. In spite of better heat detection, the conception rate was 55.9%, which is lower than during the survey phase. This could be attributed to lower fertility of semen from certain bulls used in the second phase. Assessment of progesterone values in the samples showed that 3.8% of AI were done during anovulatory oestrous cycles, 7.8% in anoestrous cows and 5.9% in cows with irregular or short oestrous cycles.

1. INTRODUCTION

According to recent estimates Myanmar has 9.7 million cattle and 2.1 million buffaloes. The proportion of cows used for dairy purpose constitutes only 10% of the total population. At present the number of artificial inseminations (AI) done range from 70 000 to 80 000 per year. AI is performed in 35% of the total diary cow population.

Use of fresh semen for AI was introduced in 1967 but has never proved to be popular with farmers, due to the low conception rate (CR) obtained and the limited choice of breeds. It was in 1976 that, with the introduction of frozen semen of European breeds, AI became population among farmers. In 1981 use of fresh semen was abandoned with substitution of frozen semen throughout the country. During the World Bank financed Livestock Development Project (1976–1981), a total of 150 000 doses of imported semen were introduced from North America and Europe. Fertility resulting from AI using imported semen was recorded at around 57% [1].

After the World Bank Project, imported semen was gradually substituted with locally produced semen. The conception rate in general was acceptable, but there were occasional decreases in fertility, most likely due to fluctuation in the semen quality. However, a thorough investigation of the causes of failure has never been carried out. The Joint FAO/IAEA Division's Co-ordinated Research Programme based on radioimmunoassay (RIA) for measuring progesterone to improve efficiency and quality of AI provided a good opportunity to address this problem in Myanmar.

2. MATERIAL AND METHODS

2.1. Location and farms

The research activities were carried out in Mandalay Division located in the central part of Myanmar. It is situated in the dry and arid zone of Myanmar where annual rain fall ranges between 1000–1500 mm. Mandalay is also the most developed area in cattle raising both for dairy as well as draught purpose. Most dairy cattle are Holstein-Friesian crosses but some villages still maintain a few local Zebu (Pya Zein) for milk purpose. Most of the dairy cattle are well fed and well managed.

Two studies were conducted, comprising a survey phase and an intervention phase. The first was conducted from February 1995 to December 1997 and the second from March 1998 to September 1998.

2.2. Survey phase

The survey was aimed at collecting samples and data from 500 cows from smallholder farmers. For this purpose, five inseminators were selected and each was assigned to sample 100 cows during the survey phase. They were asked to fill up the survey forms which had been developed for this programme when they first visited a farm, and for each cow inseminated. Milk samples were collected on the day of AI (day 0) and on days 10–12 and 22–24 after AI and were sent to RIA laboratory in Yangon for analysis. Pregnancy diagnosis was performed by the technicians themselves, between 50–150 days after AI. Milk samples were not collected regularly on day of pregnancy diagnosis during the first phase.

2.3. Intervention phase

This phase was undertaken to correct some of the drawback of the AI service that had been identified during the survey phase. The aim was to collect samples and data from 250 cows from smallholder farmers. The procedures for collecting samples and data were as described previously.

Firstly, the nutritional status of cows was improved during the pre and post partum periods in an effort to reduce the interval from calving to resumption of estrous cycles. This was done by supplementation of the feed with UMMB. The composition of the UMMB was: molasses = 35%, rice bran = 33%, urea 12%, cement = 5%, salt = 5%, and lime = 10%. Each cow was given approximately 320 g of UMMB per day (each block of 1.6 kg for 5 days). Supplementation commenced before parturition and was continued until they conceived again.

Secondly, AI technicians were briefed on the findings of the previous survey with regard to those cows which had shown high concentration of progesterone at the time of AI, and were provided with instructions and guidelines for avoiding the performance of AI during the luteal phase and other inappropriate times.

2.4. Processing of samples and data

Milk samples (10 mL) were collected by stripping of the udder, at the time of the AI technician's visit, in to vials containing a tablet of preservative (Sodium Azide). The samples were centrifuged at 2000 G for 15 min within 2hr to 3 days after collection. The skim milk fraction was transferred to another vial and stored frozen (–20°C) until assay.

Progesterone was measured in skim milk using a direct solid-phase method employing ^{125}I labelled progesterone as tracer. All samples and standards were run in duplicate. After adding standards, QC, samples and tracer, all tubes were incubated for 3 hours at room temperature. The tubes were then decanted and counted for 60 sec in a single well Gamma Counter. Counter efficiency was 63.5% and the intra-assay coefficient of variation ranged from 3.1 to 5.2%.

Information relating to the farms, cows, inseminations, AI technicians, semen batches, pregnancy diagnoses and progesterone results were recorded in the artificial insemination database application (AIDA, FAO/IAEA, Vienna) and reports were generated using the application.

3. RESULTS

3.1. Survey phase

3.1.1. Farm information:

There were 50 villages or regions involved in the survey, with distances from the AI center varying between 0–9 Km. Most of the farms were smallholdings, with 1–12 breedable females per farm. There was only one large farm, with 35 breedable females. Cows were milked 1–2 times per day and calves were weaned between 1–9 months of age.

3.1.2. AI technicians and semen batches:

The 5 AI technicians were aged between 39–55 years, had been doing AI for 10–23 years and were performing 50–80 AI per month. Formal training for all AI technicians was less than one month. Four of them had received training as veterinary assistants for two years and one was a veterinary graduate.

There were 51 batches of semen from 11 bulls. Semen dose ranged from 10–35 million live sperms per straw. Motility before freezing ranged from 60–80% and after freezing from 35–39%. The minimum standard required by the AI service is 35% post-thaw motility.

3.1.3. Cow information and fertility indices:

There were 435 cows recorded in the study, varying in parity from 1 to 8. Calving dates were between 9 February 1995 and 29 December 1996. Calving weights ranged from 204–652 kg and body condition score (BCS) from 2–5 on a 5 point scale. Inseminations were recorded starting 1 August 1996. The interval from calving to AI varied from 20–315 days. Services were recorded up to the third insemination. Milk production ranged from 2 to 24 kg, with an average yield of 11.1 kg.

The interval from calving to first service (CFSI) was 103.6 ± 40.0 days (n = 421) and the interval from calving to conception (CCI) was 111.2 ± 56.3 days (n = 315). The CR to first service was 60.8% (n = 435), while the overall CR was 63.3% (n = 501). Since it is expected that cows which do not conceive to first service should return to a second service, there should have been about 171 repeat services. However, there were only 62 second inseminations and 3 third inseminations recorded.

3.1.4. Effect of AI Technician on conception rate:

The CR achieved by the five technicians ranged from 57–68%. There was a significant difference in CR between the two sites of semen deposition: 55.7% for deposition in the cervix and 64.5% for deposition in the uterus. The CR was 64% where the passage of the pipette was easy, whereas it was 25% when passage was difficult.

3.1.5. Effect of cow on conception rate:

Conception rate increased with advancing parity from parity 2 up to 6, and then declined at parities 7 and 8. Cows of parity 1 had lower conception rate than those of parity 2. With regard to signs of oestrus, highest conception rate (70%, n = 100) was observed with standing heat, while signs such as bellowing, mounting others and mucus was associated with CR of 62.3% (n = 332). There was no difference in CR when the vulval swelling was marked (63.2%, n = 475) or slight 64.0%, n = 25). There was a significant difference in CR when AI was done in the presence of marked uterine tone (63.7%, n = 488) compared with that when uterine tone was slight (41.7%, n = 12).

3.1.6. Effect of bull, semen source and time of AI:

Semen from the 11 bulls used gave CR ranging from 50.0% to 83.3%. Semen from the local AI station gave a CR of 60.5% (n = 362) compared with a CR of 75.9% (n = 29) for imported semen.

There was no difference in fertility when AI was performed either 6 hours or 12 hours after the first detection of oestrus (61.3%, n = 194 and 64.1%, n = 287 respectively), or in the morning or afternoon (62.3%, n = 223 and 63.6%, n = 275 respectively).

3.1.7. Progesterone data interpretation:

During the survey a total of 496 milk samples collected on day of AI (day 0) were assayed for progesterone and showed that 83.5% of AI were performed when concentration was below 1 nmol/L, indicating that these cows were at a stage other than the luteal phase, while 6.3% were done when progesterone level was above 3 nmol/L, indicating inappropriate timing. Inconclusive progesterone values (1–3 nmol/L) were recorded in 10.3% of samples.

There were 454 services with two milk samples collected on days 0 and day 10–12. The progesterone values showed that 61.9% of the cows had normal ovulatory cycles, 7.3% were either anoestrous, anovulatory or had short luteal phases and 5.3% had high progesterone in both samples indicating AI during pregnancy or with luteal cysts. Inconclusive progesterone values were recorded in 25% of samples.

All three milk samples (days 0, 10–12 and 22–24) were available for 376 services. These revealed that 55.3% of the cows became pregnant, 7.7% ovulated but failed to conceive, 1.5% had late embryonic death and 1.9% of the cases were inseminated during the luteal phase. Inconclusive progesterone values were recorded in 26% of samples

3.2. Intervention phase

3.2.1. Fertility indices:

During this phase 245 first inseminations were carried out with a CR of 55.9%. The total number of inseminations was 287 and the overall CR was 58.2%. The CFSI was 95.8 ± 24.8 days (n = 245) and the CCI was 102.2 ± 28.1 days (n = 157). These intervals, subsequent to UMMB supplementation, were shorter than those observed during the survey phase.

3.2.2. AI Technicians and semen batches:

The CR for the five technicians ranged from 50.0% to 58.1%. Eight batches of semen from 8 bulls were used during this period and the CR ranged from 49.2% to 64.3%. These results were inferior to those obtained during the survey phase and one bull in particular had an unacceptably low CR of 49.2%.

3.2.3. Progesterone data interpretation:

Progesterone measurement in 272 milk samples collected on day 0 showed that 94.1% (n = 256) of the services were performed when progesterone was low and 4% (n = 11) when progesterone was high. For 259 services two samples of milk were available for progesterone measurement (days 0 and 10–12), and revealed that 81.5% (n = 211) of AI were done during an ovulatory cycle while 3.9% (n = 10) were performed at inappropriate timing. There were 204 services with progesterone values for all three samples (days 0, 10–12 and 22–24) and the results together with clinical findings and interpretation are given in Table I.

TABLE I. DIAGNOSIS OF REPRODUCTIVE STATUS OF INSEMINATED COWS BASED ON PROGESTERONE VALUES IN THREE SAMPLES AND MANUAL PREGNANCY DIAGNOSIS

Progesterone value			Pregnancy Diagnosis	n	%	Interpretation
Day 0	Day 10–12	Day 22–24				
Low	High	High	Positive	137	67.2	Pregnant
Low	High	Low	Negative	24	11.8	Non fertilization, early embryonic mortality, post AI anoestrus
Low	High	High	Negative	6	2.9	Late embryonic mortality
Low	Low	Low	Negative	16	7.8	Anoestrus
Low	Low	High	Negative	6	2.9	Anovulatory cycle, irregular cycle
High	High	Low	Negative	3	1.5	Luteal cyst, irregular cycle
High	Low	High	Negative	2	0.9	AI in luteal phase
High	High	High	Negative	3	1.5	Luteal cyst, persistent CL
Int.	Int.	Int.		7	3.4	Inconclusive
			Total	204		

Int. = Intermediate value (1–3 nmol/L)

4. DISCUSSION

The survey done in Mandalay area shows that the dairy cattle population has an average interval of 103.6 days from calving to first service, and of 111.2 days to conception. An effort to reduce these intervals through nutritional supplementation of UMMB and therefore improve reproductive efficiency resulted in corresponding intervals of 95.8 and 102.2 days respectively. The long term cost-benefit ratio of this response needs to be studied in greater detail.

First service CR of 60.7% recorded in the survey is a very satisfactory result. In the Mandalay area routine AI services have been established since many years and in the Amarapura township AI covers over 80% of the dairy cattle population. In these areas dairy cattle are mostly Friesian crosses and because of the small herd sizes animals are well managed. Unlike in large herds where each animals receive less individual attention and care, these owners understand the behaviour of the animals well, resulting in better timing of AI. It is a well known fact that timing of AI is a critical factor for achieving high CR. The AM-PM rule recommended several decades ago for insemination which is still practised seems to be valid for achieving good conception. In the smallholder system, where the cows are tied and housed most of the time, there is no opportunity for the animals to show standing heat. The important thing is for the farmer to judge when the cow really started to come into heat. This judgment by the farmer could be ambiguous and may not coincide with the true onset of heat. The present study however shows that there is no difference in fertility when AI is done either 6 hr or 12 hr after onset of oestrus. There was also no significant difference detected between AI performed in the morning or afternoon. The results in this respect were similar during both phases of the study.

The survey confirmed that there are some cases of wrong detection of oestrus by farmers. The progesterone assay showed that 6.3% of services were performed in the luteal phase of the oestrous cycle. Wrong detection of oestrus as a major cause of poor AI performance is stated in several studies [2, 3]. The finding in the Mandalay survey however indicates the situation was not as serious as in some findings where around 20% of the animals presented for AI have high levels of progesterone [4].

In the intervention phase of this study an attempt was made to correct the faults in detection of heat by holding a workshop for the AI technicians, especially with respect to the animals presented for AI with high concentration of progesterone on day of AI. This measure was found to be effective as in the percentage of animals presented with high progesterone declined to 4%.

Assay results for the progesterone concentration during the survey showed a high occurrence of intermediate values, especially when all three samples were considered, making interpretation difficult in about 25% of cases. In the second phase of study more emphasis was placed on the proper handling, storage and shipment of milk samples. This resulted in a marked reduction in the occurrence of samples with intermediate values to around 3%. This permitted a more accurate assessment of reproductive status, and showed that 81.9% of the services were done during a proper ovulatory cycle. The occurrence of non-fertilization or early embryonic mortality accounted for 11.8% and late embryonic mortality for 2.9%. Other aberrant forms of reproductive status constituted 13.8% and included anoestrus, anovulatory cycles, luteal cysts or persistent CL.

In spite of better accuracy of heat detection the CR during the intervention phase declined slightly compared with that in the survey phase. This could be attributed to one particular batch of semen which had achieved a CR of only 49.2%. Variation in the fertility of bull semen is one of the main attributes that can alter the overall CR. Therefore, the need for special attention to test each batch of semen and to monitor the fertility results regularly is highlighted by this study. However, as discussed above, the nutritional intervention tested during this phase resulted in a reduction in the intervals from calving to first service and to conception, thereby improving reproductive efficiency.

5. CONCLUSION

The survey revealed that fertility to AI in the Mandalay region of Myanmar can be considered as good for a tropical developing country. Supplementary feeding with UMMB was effective in reducing the intervals from calving to first oestrus and to conception. Re-training of inseminators with emphasis on detection of heat and avoidance of AI during inappropriate times resulted in a lower incidence of such inseminations. An important factor found to influence fertility was the quality of semen, highlighting the importance of testing each batch for fluctuations during storage, and of regularly monitoring the fertility rates achieved by bulls used as semen donors.

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ARTIFICIAL INSEMINATION OF CATTLE IN SRI LANKA: STATUS, PERFORMANCE AND PROBLEMS

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Abstract

ARTIFICIAL INSEMINATION OF CATTLE IN SRI LANKA: STATUS, PERFORMANCE AND PROBLEMS.

Artificial insemination (AI) has been accepted as the primary breeding tool in genetic upgrading programmes of cattle in Sri Lanka. Three studies were conducted, to determine the coverage and performance of AI at national, provincial and district levels (Study 1), the success rate and factors affecting success rate of AI in wet zone mid-country smallholder farms (Study 2) and in wet zone up-country large multiplier farms (Study 3). The objective was to design, implement remedial measures and/or determine future studies necessary to improve the efficiency of AI services. Study 1 revealed that at national level the AI service reached less than 15% of the breedable cattle and accounted for less than 6% of estimated annual calvings. The coverage reached above 50% of the breedable cattle only in the wet zone while in the intermediate and dry zone areas it was negligible. Study 2 revealed that the mean calving to first service interval (CFSI) in cattle of the wet zone mid-country small holdings was 183 ± 87.1 days (n = 211) and the calving to conception interval (CCI) was 194 ± 93.9 days (n = 143). The first service conception rate (FSCR) was 45% and the overall conception rate (OCR) was 50.2%, with an average of 1.99 services per conception (S/C). Study 3 showed that the mean CFSI and CCI in wet zone up-country multiplier farmers were 111.2 ± 74.2 days (n = 133) and 156 ± 92.7 days (n = 170) respectively. The average FSCR and OCR were 50.4% and 53.6% respectively and the average S/C was 1.9. Study 1 showed that the AI coverage of the island is very low and the proportion of calvings from AI is too low to have a significant impact on genetic composition of the national cow population. Studies 2 and 3 showed that the success rate of the AI service in the more favourable and extensively covered wet zone areas was also low. These studies revealed that factors associated with the chain of events from farmer, cow, semen to the technician contributed to poor fertility.

1. INTRODUCTION

Artificial insemination (AI) could be regarded as one of the oldest and robust biotechnological applications that has made a significant impact in animal agriculture throughout the world. It was first carried out in farm animals at the beginning of the twentieth century by the Russian scientist Ivanoff and, by the 1930s, had spread to Europe and Northern America.

AI was first introduced into Sri Lanka in 1938 by T.M.Z. Mahamooth [1] and after successful trials, was offered to private breeders around Peradeniya. However, the acceptance by the breeders was very low. After independence from British rule in 1948, improvement of peasant agriculture and livestock was undertaken as a major strategy for development of the rural agrarian economy [2] and AI services commenced field operations in 1951 [1, 3]. Initially it was provided through the main centre at Peradeniya and two sub-centres in Colombo and Galle. By 1955, the number of sub-centres had increased to 17 and all were located in close proximity to railroads as it was necessary to transport processed liquid semen with minimum delay from the processing centre at Peradeniya. In 1960, semen collection and processing activities were transferred to Central Artificial Insemination Service Centre (CAIS) at Kundasale. This was followed by the establishment of two regional semen processing laboratories, one at Tinneveli and the other at Polonnaruwa. The deep frozen semen technology was introduced into the country on an experimental basis in 1966 and came into commercial operation in 1968 [4]. By 1975, there were 65 AI sub-centres in operation, spread across most regions of the country except the dry zone. Extension to the dry zone was gradual, and by 1997, there were 168 Veterinary Offices with 137 AI sub-centres operating the service with 357 technicians (258 government technicians and 99 private technicians). The number of AI services per annum increased from 72 in 1951 to around 108 338 in 1996. This expansion of the AI service was supported by several international donor programmes [5].

Although the AI service is in operation on an island wide basis, few scientific studies have been conducted to assess its performance [6, 7]. One study [6] reported that the efficiency of heat detection by smallholder farmers was less than 65% and nearly a third of inseminations were carried out during the wrong time with respect to oestrus. The other [7], a limited scale study in the wet zone mid-country area, showed that the conception rate (CR) was lower than expected and suggested that this was most likely due to poor heat detection by the farmers and delays in getting the cow served. Two consultancy reviews [8, 9] have also highlighted the poor performance of the AI service at national level.

Today the challenges to the farmer, extension worker and livestock researcher are many. The current level of productivity of livestock is too low to retain resources (land, labour and capital) in animal agriculture. Yet the rural resource poor have no skills to venture into any other economic pursuit. As such they will continue to remain in peasant-based agriculture and continue to perform poorly unless interventions are introduced. Therefore the need at this crucial juncture is to improve the productivity of animal agriculture so as to sustain the rural economy and maximise the welfare of the society. Within animal agriculture, the dairy sector forms the largest component. Its productivity has to increase several-fold in order to sustain this valuable industry and genetic improvement of the national cattle population is an obligatory step towards this goal. Today AI is recognised as the primary tool for genetic improvement in cattle breeding. Therefore, a series of studies was undertaken, to determine the coverage and performance of AI at national, provincial and district levels (Study 1), the success rate and factors affecting it in wet zone mid-country smallholder farms (Study 2) and in wet zone up-country large multiplier farms (Study 3). The objective was to design, implement remedial measures and/or determine future studies necessary to improve the efficiency of AI services.

2. MATERIALS AND METHODS

2.1. Study 1: Assessment of national, regional and district level coverage and performance of AI services [10]

2.1.1. Data collection

The records at the Division of Animal Breeding in the Department of Animal Production and Health (DAPH) and the Division of Planning and Monitoring in the Ministry of Livestock Development and Estate Infrastructure (MLDEI) were examined and the following data were extracted for the country, agro-ecological zones, provinces and districts: i) total number of AI service centres; ii) total number of cattle; and iii) number of AIs and recorded calvings. The published reviews and consultancy reports available at the data bank of DAPH were also reviewed.

2.1.2. Data analysis

A map of Sri Lanka depicting the agro-ecological zones, provincial boundaries, districts, locations of semen processing centres, veterinary surgeon's (VS) offices providing AI service and AI sub-centres centres was drawn up. Estimates of total breedable cattle and annual calvings at national, provincial and district levels from 1983 to 1996 were calculated. Percentages of breedable cattle covered by AI and proportion of estimated calvings due to AI at national, provincial and regional levels were calculated. The same data was rearranged according to three major agro-ecological zones of the country. For analytical purposes, the following assumptions were made: i) all recorded AIs were considered as first services, ii) 60% of the total cattle population were considered as females, iii) 55% of the females were considered as breedable; and iv) the annual calving rate of the breedable cattle population was considered as 50%. These assumptions were applied across the island, agro-ecological zones, provinces and districts.

2.2. Study 2: Survey on success rate of AI in smallholdings of the wet zone mid-country region [11]

2.2.1. Location

The study was conducted in the wet zone mid-country region since it is the one where the highest percentage of cattle belonging to smallholdings are bred artificially. It is located between 600–800 metres above sea level, with an annual rainfall of 2000–3000 mm, temperature of 25–28°C and humidity of 75–80% during the year. The dairy production system is characterised by smallholdings (1–2 cows/herd) of *Bos taurus* genotype, managed under zero grazing with natural grasses and supplemented with limited quantities of commercial concentrate feed. Five VS ranges (Gampola, Kundasale, Teldeniya, Udunuwara, and Yatinuwara) from this region were purposively selected for the study.

2.2.2. Longitudinal study

A sample of cows was monitored by performing a longitudinal field study during the period January 1996 to June 1997. In liaison with the VS offices and the inseminators serving these five ranges, dairy cows receiving first inseminations following a recorded calving were monitored until they were confirmed as pregnant. One investigator accompanied the inseminator to smallholder farms when there was a call for an AI. At the time of AI (day 0), the following detailed information relating to the farm, cow, semen and inseminator were recorded.

2.2.3. Farm data

Information was recorded regarding total land extent, herd size, the type of management, feeding systems and breeding practices, including the voluntary waiting period from detection of oestrus to insemination, housing system and herd composition.

2.2.4. Cow data

For cows presented to AI the identification number, date of birth, parity, last calving date, last calving type, date of first postpartum heat, body weight, service number, dates of AI, interval between heat detection and AI, time of day at which AI was done, observed heat signs, degree of vulval swelling, colour of mucous discharge, degree of uterine tone, site of semen deposition, code of semen, lactation state, body weight, body condition score (BCS) at time of AI and average daily milk yield were recorded.

2.2.5. Semen/bull data

For each AI performed the breed of semen donor, identification number, source of semen (local/imported), volume, type of semen (chilled/frozen), quality of semen (if available) and sperm dose were recorded.

2.2.6 AI technician data

Information on age, highest level of education, duration of formal training in AI, years of experience, average number of AI per month, type of employment, method of thawing semen and other non-AI work performed were recorded for each technician participating in the study.

2.2.7. Milk sampling for progesterone measurement

On day 0, a milk sample (10 mL) was collected into a bottle containing a potassium dichromate tablet as preservative. A second milk sample was collected 10–12 days later from the same cow and, if the cow was not presented for a repeat AI, a third milk sample was collected at 21–23 days after the AI. Dates of subsequent services if any were recorded for cows presented for repeat services. In all cases, those not returning to service within 60–90 days after the last service were examined for pregnancy by rectal palpation and the findings were entered in record sheets.

2.2.8. Milk progesterone assay

Milk samples were placed in a refrigerator (4°C) within 6 hours of collection and transferred to the laboratory for processing within 7 days. They were centrifuged at 4°C and 1000 × g for 10 minutes, the fat-free fraction (skim milk) was drawn off and stored at –15°C until assayed for progesterone using a direct solid-phase radioimmunoassay (RIA) employing antibody-coated tubes, ^{125}I-progesterone as tracer and standards (0, 1.25, 2.5, 5, 10, 20 and 40 nmol/L) prepared in skim milk (kits supplied by the FAO/IAEA Sub-programme on Animal Production and Health, Vienna). The intra-assay and inter-assay coefficients of variation were 9% and 14.5% respectively.

2.2.9. Data tabulation and analysis

Data were recorded and partially analysed using a computer database named AIDA (Artificial Insemination Database Application) which was developed by the FAO/IAEA Sub-programme. For further analysis data was exported to Systat (V.6.0 for Windows, SPSS).

2.3. Study 3: Survey on success rate of AI in state multiplier farms [12]

2.3.1. Location

Four multiplier farms, namely Ambewela, Bopaththalawa, Dayagama and New Zealand, were selected purposively for the study. They are located at 2000 metres above sea level, with an annual rainfall of 2000–2500 mm distributed throughout the year. The mean temperature ranges from 10°C in December to 25°C in April, with relative humidity of 75–80%. The dairy production system is based on pure *Bos taurus* genotypes, managed under zero grazing in one farm (New Zealand) and daytime grazing with stall-feeding at night in the other three. Feeding of commercial concentrates and mineral mixture is done on all farms.

2.3.2. Longitudinal study

Following the same procedures described in Section 2.2, two hundred cows receiving first insemination following calving were monitored during the period from April 1997 to December 1997. A sample of 200 cows bred artificially were studied longitudinally. The recording of data, collection and analysis of milk samples, and the recording and analysis of data were done as described previously.

3. RESULTS

3.1. Coverage and performance of AI service

A map of Sri Lanka depicting the agro-ecological zones, provincial and district boundaries, spread of semen processing centres, VS offices and AI sub-centres, total land extent and cattle population is given in Fig. 1. The total land extent of the island is 65 610 sq. km and the dry zone accounts for 62.9% of the total land area, while the intermediate and wet zone accounts for 13% and 24.1%, respectively. The total number of cattle is estimated at 1.7 million with 48%, 36.7% and 15.3%, respectively in dry, intermediate and wet zones. The largest concentration of AI service is in the wet zone region, followed by intermediate zone with the lowest coverage in the dry zone. The cattle density is lowest in the dry zone, followed by the intermediate zone with the highest concentration in the wet zone. Administratively the country is divided into 7 provinces; Central province (CP), Western province (WP), Southern province (SP), Eastern province (EP), Northern province (NP), North-western province (NWP), North-central province (NCP) and Sabaragamuwa province (Sab.P). The total number of administrative districts and divisions are 25 and 302 respectively and are served by 194 VS offices and 207 AI sub-centres (Internal Reports of the Department of Animal Production and Health).

3.1.1. At national level

The total number of AI performed in the country rose from 47 318 in 1983 to 109 008 in 1996 and the number of recorded calvings from AI rose from 2724 in 1983 to 18 183 in 1996. The increase in the total number of services paralleled the expansion of the veterinary service across the country, provinces and districts.

	Agro-ecological Zone	Land extent (Sq.km)	Number of Cattle	Density of cattle population (No./sq.km)	Command area/VS office/AI sub-centre (Sq.km)	Number of breedable cattle/VS Office/AI sub-centre
	Wet zone	41,300	844,200	20.4	555.0	3714
	Int. zone	8,500	646,600	76.1	71.8	1778
	Dry zone	15,810	269,200	17.0	177.6	998

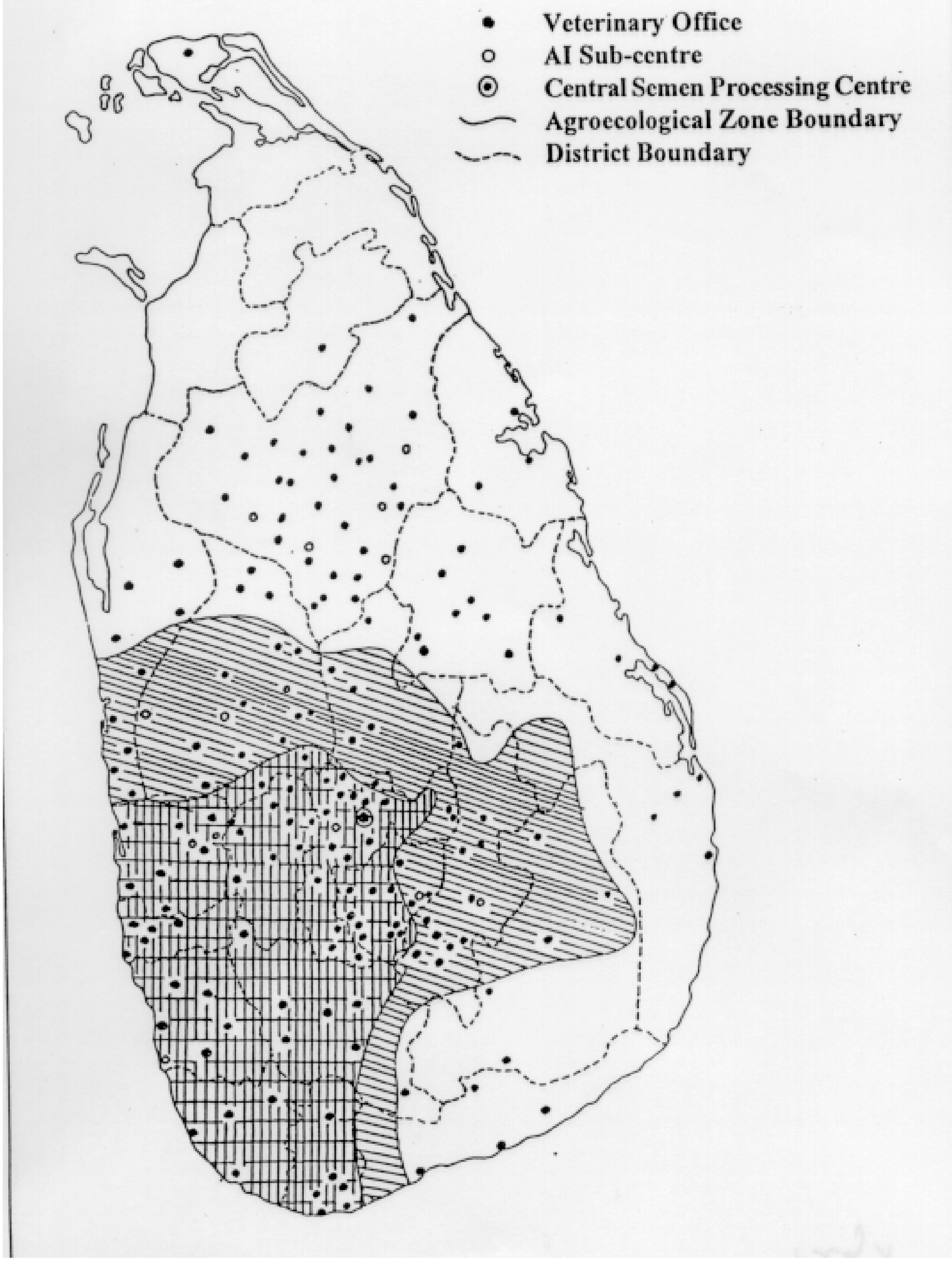

FIG.1. Map of Sri Lanka depicting the agro-ecological zones, district boundaries and locations of artificial insemination service points (veterinary offices and AI sub-centres).

The percentage of breedable cows covered by AI gradually increased from 8.6% in 1983 to 18.8% in 1996 and the proportion of AI calvings from the total estimated calvings rose from less than 1% in 1983 to 6.3% in 1996. The number of estimated female calves produced from AI in 1996, assuming that 50% calvings result in female calves and with 5% calf mortality was estimated to be 8642. The number of male calves produced appears to be less than this because of higher mortality among male calves. Empirical evidences suggest that only a small percentage of male calves reach adulthood and of them very few enter into the breeding population.

3.1.2. At agro-ecological zone level

The total number of AI done, coverage of breedable cows and performance criteria during 1996 for the wet, intermediate and dry zones are given in Table I.

TABLE I. NUMBER OF AI, COVERAGE AND PERFORMANCE IN DIFFERENT AGRO-ECOLOGICAL ZONES IN 1996

Agro-ecological Zone	Number of breedable cattle	Number of AI performed	Percentage of Breedable cows served by AI	Number of Recorded AI calvings	Calving rate from AI (%)	Number of estimated calvings	Percentage of calvings due to AI
Wet	88 836	60 913	68.6	12 228	20.1	44 418	27.5
Intermediate	213 378	35 989	16.9	4 616	12.8	106 689	4.3
Dry	278 586	12 106	4.3	1349	11.1	139 293	<0.1
Total	580 800	10 9008	18.8	18 193	16.7	290 400	6.3

Source: Department of Animal Production and Health (1997)

In 1996, the wet zone had recorded the highest number of inseminations, the highest coverage in terms of the proportion of breedable cows served by AI, highest number of calvings due to AI and the highest percentage of estimated calvings due AI. The dry zone had the lowest figures for all these parameters.

3.1.3. At provincial level

The total number of AI done, coverage of breedable cows and performance criteria during 1996 at provincial and district levels are shown in Table II. The highest number of AIs were done in CP and the lowest were recorded in EP and NP. The coverage in terms of the proportion of breedable cattle was highest in CP followed by WP, Sab.P, NWP, NCP, UP and SP. Lowest coverages were recorded in EP and NP. The highest calving rate was recorded in WP, followed by SP, CP, Sab.P, NWP, UP and NCP. The percentage of total estimated calvings after AI was highest in CP, followed by WP and was low in Sb.P, NWP, SP, UP and NCP. The proportion of calvings from AI was negligible in EP and NP.

3.1.4. At district levels

As shown in Table II, the coverage in wet zone districts was relatively high and ranged from about 10% in Matara to 100% in Kandy and Nuwaraeliya. In the intermediate zone areas the coverage was relatively low, lowest being in parts of Hambantota and Moneragala and high in Badulla. In most of the dry zone the coverage was very low (less than 0.1%) except in Anuradhapura, Polonnaruwa and Puttalam. The success rate as measured by the calving rates ranged from 30.4% in Gampaha to negligible levels in districts of EP and NP. Highest proportion of calvings from AI was recorded in Nuwaraeliya, followed by Kandy, Gampaha and Kegalle with other districts recording low percentages. Most wet zone districts recorded high proportion of calvings due to AI (>15%) while the intermediate zone areas recorded low proportions (<10%) with negligible levels in all dry zone districts except in Polonnaruwa and Anuradhapura districts which recorded around 3%.

TABLE II. NUMBER OF AI, COVERAGE AND PERFORMANCE IN DIFFERENT PROVINCES AND DISTRICTS IN 1996

Province	District	Total AI	Percentage coverage	Calving rate following AI	Percentage calvings due to AI
Central province	Kandy	21 744	100	18.8	45.9
	Matale	2831	20.3	14.7	4.5
	N'Eliya	15 182	100	28.1	49.9
Sub-total		*39 757*	*88.1*	*21.7*	*34.3*
Western province	Colombo	2227	28.1	12.2	6.7
	Gampaha	11 253	66.2	30.4	37.5
	Kalutara	2639	19.4	25.2	6.8
Sub-total		*16 179*	*42*	*26.6*	*17.8*
North western province	Kurunegala	15 600	23.4	13.4	4.5
	Puttalam	4560	14.1	29.2	6.4
Sub-total		*20 160*	*20.3*	*17.1*	*5.2*
North central province	Anuradhapura	7075	15.1	11.6	3.2
	Polonnaruwa	3077	17.5	11.2	3.1
Sub-total		*10 152*	*15.7*	*11.5*	*3.2*
Sabaragamuwa province	Kegalle	4058	54.4	23.4	24.7
	Rathnapura	1420	10.4	7.4	1.3
Sub-total		*5478*	*26*	*20.6*	*8.9*
Southern province	Galle	3810	32.5	18.7	7.8
	Matara	1147	18.4	45.5	11.3
	Hambantota	763	2.4	<0.1	<0.1
Sub-total		*6020*	*11.4*	*22.1*	*4*
Uva province	Badulla	8726	33.3	13.5	7.5
	Moneragala	642	3.6	<0.1	<0.1
Sub-total		*9368*	*12.4*	*11.5*	*4.5*
Eastern province	Ampara	528	1.4	29.5	<0.1
	Batticoloa	230	<0.01	<0.1	<0.1
	Trincomalee	331	<0.01	<0.1	<0.1
Sub-total		*1089*	*<0.01*	*18.6*	*<0.1*
Northern Province	Jaffna	214	<0.01	<0.1	<0.1
	Kilinochchi	0	<0.01	<0.1	<0.1
	Mannar	0	<0.01	<0.1	<0.1
	Mulativu	0	<0.01	<0.1	<0.1
	Vavuniya	652	<0.1	30.1	<0.1
Sub-total		*865*	*<0.1*	*2.8*	*<0.1*
Island total		*109 008*	*18.8*	*16.7*	*6.3*

Source: Department of Animal Production and Health (1997)

3.2. Success rate of AI in smallholdings

3.2.1. Fertility

The fertility indices of cattle subjected to AI in four veterinary ranges, based on 211 first inseminations on smallholder farms, are given in Table III.

TABLE III. FERTILITY INDICES IN CATTLE SUBJECTED TO AI IN FOUR VETERINARY RANGES IN THE MID-COUNTRY WET ZONE OF SRI LANKA

Veterinary	Mean (± SD) interval (days) from calving to:		Conception rate (%) to:		Services per
Range	First service	Conception	First service	All services	conception
Gampola	131 ± 51.5 (n = 34)	113 ± 50.6*(n = 17)	50 (n = 34)	50 (n = 34)	2
Kundasale	170 ± 114 (n = 52)	188 ± 74.8 (n = 39)	34.6 (n =52)	45.9 (n = 85)	2.2
Udunuwara	186 ± 80.8 (n = 84)	200 ± 89.3 (n = 73)	53.6 (n = 84)	60.8 (n = 120)	1.6
Yatinuwara	242 ± 103.3 (n = 45)	265 ± 131 (n = 15)	33.3 (n = 45)	31.3 (n = 48)	3.2
Overall	183 ± 87.1 (n = 211)	194 ± 93.9 (n = 143)	45 (n = 211)	50.2 (n = 287)	1.99

* Based on first service conceptions only

The average calving to first service interval (CFSI) was well over 6 months and the average calving to conception interval (CCI) was nearly 200 days, with significant differences (P <0.05) due to location in both parameters. Cows at Yatinuwara VS rage recorded longest CFSI and CCI while the cows at Gampola VS range recorded the shortest CFSI and CCI. Similarly, first service conception rate (FSCR) and the overall conception rate (OCR) were significantly different (P <0.05) among three locations; with Yatinuwara recording the lowest and Udunuwara recording the highest values. At the time of the first postpartum service, the mean body weight of the cows was 321.2 ± 77.5 kg, the body condition score (BCS, on a 1–5 scale) was 2.7 ± 0.4, and the average milk production was 7.7 ± 4.1 L per day.

3.2.2. Interpretation of progesterone data

Results from milk progesterone assay for day 0 samples (n = 258) revealed that 79.4% of the AIs were done when progesterone was below 1 nmol/L, indicating that the cow could have been in heat, but 13.2% of AIs were done when progesterone was above 3 nmol/L, when the cow could not have been in heat. The remainder of the samples (8.5%) had values in the inconclusive range (>1 and <3 nmol/L). Progesterone values for samples collected on days 0 and 10–12 (n = 258) showed that only 64.3% of the animals had a normal ovulatory cycle, while 10.5% were in anoestrous, 5% were pregnant and 5.4% were in luteal phase. Intermediate progesterone values were found in 14.7% of samples. The progesterone values from all three samples (days 0, 10–12 and 21–23) were available for 237 services and these along with data from pregnancy diagnosis (PD) by rectal palpation 60–90 days after service indicated that 44.3% conceived to that service. In 8.9% AI was done in the follicular phase but they failed to conceive or had early embryonic death and were not detected in heat until PD was done. Late embryo mortality or persistent corpus luteum (CL) occurred in 3.8%. In 2.1% of cases AI had been done during pregnancy while 6.3% were done in anoestrous cows. Intermediate level of progesterone with doubtful rectal findings at 60–90 days post-service was found in 23.6% while in 11% the results were not physiological meaningful.

The overall analysis of progesterone data revealed that 25.5% of AIs were performed at an erroneously detected heat. Of these, 13.2% were done in cows with an active CL where nearly half of them were pregnant at the time of AI, while 12.3% were done in anoestrous cows. Nearly 20% of the cows submitted for PD were found to be non-pregnant, while 5% of the cows which became pregnant had subsequently suffered late embryonic mortality.

3.2.3. Effect of different factors on success of AI in smallholder farms

Farm factors: No differences in CRs were evident in animals managed semi-intensively and intensively. However, tendency for higher, but statistically non significant, CRs were observed in animals fed concentrates (40%) than in those not fed concentrates (27%). When farms were grouped according to percentage of family income from dairy farming, those with less than 25% had an average CR of 26%, while those with higher proportions had CRs above 40%, but this difference was not statistically significant. For AIs performed 6, 12, 18, 24 and 30 hours after first detection of heat, the CRs were 25, 34.7, 38.1, 68.4 and 37.5%, respectively. The highest CRs (>60%) were recorded during the months of June, July, August and September, while the lowest (<30%) were recorded in March, April and November.

Cow factors: A tendency was seen for declining CRs from parity 1 up to a parity of 5; thereafter the numbers were too small for comparisons. Cows receiving their first service before 60 days postpartum had lower CR (P <0.05) than those receiving the first service after 60 days. The data on other cow factors such as breed, body weight, body condition, milk yield at AI and intensity and type of oestrous signs were inconclusive.

Bull/semen factors: Semen from 22 bulls had been used in the sample under study. Of these, semen from seven bulls had been used for more than 20 services and their CRs ranged from 18.2–70.4% (P <0.05). Semen originating at the Kundasale AI station gave higher (P <0.05) CR (49%, n = 181) than imported semen (30%, n = 166). Data on effects of volume and dose of semen, type of semen (chilled or frozen) and motility before processing was inconclusive.

Inseminator: In the present study, only one technician was monitored in each of three veterinary ranges (Gampola, Kundasale and Udunuwara), while two were monitored in the fourth range (Yatinuwara). Technician had a significant effect (P <0.05) on conception rate, with the overall CRs achieved by them ranging from 27.8% in Yatinuwara to 58.5% in Udunuwara. However, the technician factor is confounded within location and hence true effects cannot be separated.

3.3. Success rate of AI in large farms

3.3.1. Fertility

As shown in Table IV, the overall average CFSI and CCI were over 4 and 5 months, respectively. The longest CFSI was recorded at New Zealand farm while the shortest interval was at Dayagama. The longest CCI was recorded at New Zealand farm while the shortest was at Bopaththalawa. Both the FSCR and OCR were lowest at New Zealand farm and highest at Bopaththalawa farm

TABLE IV. FERTILITY INDICES OF CATTLE SUBJECTED TO AI

Farm	Mean interval (days) from calving to		Conception rate (%) to		Services per Conception
	First service	Conception	First service	All services	
Ambawela	102 ± 63.2 (n = 13)	159 ± 103.6 (n = 23)	53.8 (n = 13)	65.7 (n = 35)	1.5
Bopaththalawa	109 ± 66.3 (n = 34)	136 ± 65.6 (n = 42)	70.5 (n = 34)	69.3 (n = 62)	1.4
Dayagama	91 ± 33.5 (n = 35)	148 ± 101.5 (n = 45)	57.1 (n = 35)	60 (n = 75)	1.7
New Zealand	128 ± 96.5 (n = 51)	175 ± 96.1 (n = 60)	31.3 (n = 51)	40.8 (n = 147)	2.5
Overall	111.2 ± 74.2 (n = 133)	156 ± 92.7 (n = 170)	50.4 (n = 133)	53.6 (n = 319)	1.9

3.3.2. Interpretation of progesterone data

Results from milk progesterone assay for day 0 samples (n = 199) revealed that 79.4 % of the AIs s were performed when progesterone concentration was below 1 nmol/L indicating that the cow could have been in heat, but 9.5% of AIs were done when progesterone concentration was above 3 nmol/L, indicating that the cow could not have been in heat. The remainder of the samples (11.1%) had values in the inconclusive range (>1 to < 3nmol/L). Progesterone values of samples collected on day 0 and 10–12 (n = 199) showed that only 67.3% of the animals had normal ovulatory cycles. In 4.5% of cases AI was done in anoestrous cows, in 5.5% cases in pregnant cows and in 2.0% during the luteal phase. Intermediate progesterone values were found in 20.6% of the samples.

The progesterone values from all three samples (Days 0, 10–12 and 21–23) were available for 166 services. Progesterone data along with rectal palpation findings at PD indicated that 50% had conceived; 6.6 % most likely had non-fertilisation or early embryonic death or had gone into post-AI anoestrous; 4.2% had late embryonic death, luteal cyst or persistent CL; 1.2% were inseminated during pregnancy; and 2.4% were inseminated during anoestrus. None of these animals were detected in heat until PD. Intermediate progesterone values and inclusive rectal findings were found in 26.5% of the animals.

Overall analysis of progesterone data suggested that 16.4% of the AIs were done at an erroneously detected heat. Of these, 13.2% were done in cows with an active CL where more than half of them were pregnant at the time of AI.

3.3.3. Effect of different factors on AI

Farm factors: The farm had significant effect (P <0.05) on the CR, with highest OCR observed at Bopaththalawa farm and the lowest at New Zealand farm. Farms which coupled visual heat detection with use of teaser bulls had higher CRs (64.5%) than those using only visual detection (40.8%). When the time from heat detection to AI was grouped as 0–6, 6–12, and 12–24 h, the respective CRs were 60%, 49.2% and 51.4%. Higher CRs (>50%) were recorded during the months of

August, June, July and September while the lower CRs (<40%) were recorded in April and March. But neither the time of AI nor the time of the year effect was statistically significant

Cow factors: There was a significant breed effect (P <0.05) on CR. Among the pure breeds kept in these farms the Ayrshire breed had the highest CR (Ambewela, 65.7%), with the Jersey breed intermediate (60%) and the Friesian breed having the lowest (New Zealand, 40.3%). However, the breed effect is compounded within farms and hence true effects cannot be delineated. Parity had significant effect (P <0.05) on CR, with a steady increase from parity 1 to 6 followed by a decline to the lowest CR in parity 10. A tendency for higher CRs were seen in animals with BCS of 2.5 and above and lower CRs in those with BCS below 2.5, but this was not reflected in the pooled data across farms. Type of heat sign noted at the time heat detection or service had significant effect (P <0.05) on CR; cows which were observed to be at 'standing heat' at the time of AI had the highest CR (64.2%, n = 81) whereas those with signs of 'mounting others' and 'mucus discharge' had CRs of 47.4% and 42.5%, respectively.

Bull/semen factors: Imported deep frozen semen from five donor bulls (Ayrshire, Friesian and Jersey) were used on the cows in this study. The resulting CRs ranged from 34.2 to 68.9% with significant difference (P <0.05) among donors; semen identification number Fr-390-1997 had achieved the highest CR (68.9%, n = 61) while the semen identification number Fr-387-1997 achieved the lowest CR (34.2%, n = 114). Semen code numbers Jr-208-1997, Fr-388-1997 and Ay-85105 had CRs of 59.5% (n = 74), 59.1% (n = 22) and 63.6% (n = 33), respectively.

Inseminator related factors: Three farms (Ambewela, Dayagama and New Zealand) had one AI technician per farm while the other (Bopaththalawa) had 2 technicians and all were resident on the respective farms. The technician factor was significant, with CRs ranging from 40.6% for the technician at New Zealand farm to 72.3% for one at Bopaththalawa. However, as the technician factor was confounded within farm factor, true effect of technician cannot be isolated.

4. DISCUSSION

4.1. AI service at national level

Although AI has been considered as the primary tool for cattle breeding in the country, it still reaches less than 20% of the breedable cattle population and accounts for less than 10% of the annual calvings in the country. Considering that only half of the calves born are females, coupled with the known high rate of wastage of male calves, the infusion of new genetic material into the national cattle population is therefore extremely low.

It was evident that although the AI service is in operation throughout the island, this service has gained grounds and established itself as a primary breeding technique only in a few provinces. In terms of numbers of cattle only about 10% of the estimated breedable cattle population (approximately 600 000 cattle) is provided with a coverage above 50%. The rest of the population (90%) are provided with very marginal coverage and more than two-thirds are still be bred naturally, mostly by scrub bulls.

This low level of coverage on a national basis as well as in some areas of the country appear to be due to many factors. The most significant factor is the variation in the infrastructural facilities, which is closely related to land use patterns coupled with the agro-ecology.

In the dry zone, which covers nearly two-thirds of the land mass and carries nearly half the cattle population, the coverage is less than 10% with a negligible level of coverage in many areas, particularly in the NCP, UP, SP, EP and NP. These areas have remained underdeveloped with respect to roads, transport and communication due to their low population density and harsh climatic conditions. Cattle are reared under extensive systems with dependence on communal grazing grounds which are often located far away from the farmers' dwellings. Further, the predominant genotypes are indigenous and the expression of heat signs is not well marked. Farmers in these areas rely on traditional knowledge rather than on modern technologies. This creates a problem for AI services as the intended recipients are not well motivated to get the benefits of such a service and are unable to detect heat signs and get the animals served in time. This clearly shows that the farmers need to be educated. However, the relatively few veterinarians in such areas are unable to fulfil this task due to the low resources in terms of trained personnel, mobility and operational budgets at their disposal. Most parts of the intermediate zone too have similar problems. Though the infrastructural facilities are relatively better compared with the dry zone, farmers in this zone are not willing to invest time and labour for an activity which does not bring enough returns. It is well known that the milk market is heavily distorted and the producers lack bargaining power at the present time. The combined effect of all these factors is the low AI coverage of animals in the dry and intermediate zones of the country.

In the wet zone areas with relatively better infrastructure and reasonably well informed farmers, such as in CP and WP, the majority of the animals are covered by AI. In these areas the predominant genotype is *Bos taurus* pure or crosses, the herd size is small, animals are managed with more care and farmers are compelled to use AI due to the absence of bulls for natural mating. Yet the success rate is lower than it ought to be.

The disparity in coverage and also the poor performance of AI in the different agro-ecological zones poses a serious question to development workers in the livestock sector. AI has been declared as the primary breeding tool in efforts towards genetic up-grading. However, in the light of continuous poor coverage and performance, particularly in most areas of the dry and intermediate zones, this policy may need to be re examined. This concern has also been expressed in many previous consultancy reports [8, 9].

4.2. Smallholder farms

In about a quarter of smallholdings in the mid-country wet zone of Sri Lanka, cattle farming for dairy production is an integral component of a mixed farming system [13]. The animals are predominantly *Bos taurus* genotypes and the system of management is semi-intensive or intensive. Most farmers resort to AI for breeding their cows, due either to the awareness of the value of AI for obtaining a calf of higher dairy potential or the unavailability of good quality stud bulls in their villages. The present study revealed that the majority of farmers had no specific preferences for breeds or type of semen indicating their lack of appreciation of the relative merits and suitability of different breeds for their particular farming environment. This points to an important need to educate the farmers on breeding goals and the appropriate use of AI to achieve these goals. The calving to first service interval was well over 4 months and this may most likely stem from the poor knowledge of farmers on reproductive management of cattle. The majority did not breed their cows until several months after calving because of the misconception that early re-breeding adversely affects the milk yield of the cow as well as the growth of the calf. The average calving to first service interval in this study was 183 days, whereas in a previous study in this area [14], the average interval from calving to first rise in plasma progesterone was around 75 days. While one of the reasons for the delay in first service could have been postpartum anoestrous due to poor nutrition as evident from the study, it is also likely that even those which returned to oestrus early in the postpartum period were kept unbred by the farmers due to the above misconceptions.

As evidenced from progesterone measurement in milk samples, the accuracy of oestrous detection has been significantly low, with 13.2% having an active CL and 12.3% being anoestrous at AI. Even in those with low progesterone levels at AI, only 64% appeared to have had a normal ovulatory oestrus. There is no doubt that these errors in oestrous detection would have contributed significantly to the observed low success rates of AI in this study. These results reinforce the findings of previous studies [6, 7] and indicate the need for farmer education, training and extension in this area.

The average first service CR was 45%, which can be considered to be within the lower range of acceptability for a developing country. However, two of the veterinary ranges studied had CRs below 35%, which is below acceptability. A large number of factors relating to the farm, cow, semen and inseminator were recorded in this study. However, given the highly heterogeneous nature of the sample, many of these factors did not have sufficient records in their different categories to allow for accurate interpretation. Also, several of the factors were interrelated and therefore produced confounding effects. However, a few factors, as discussed below, were perceived to be of practical importance and were found to have an overriding influence on CRs. These provide insights to ways in which the fertility of cattle in this region, as well as the AI service, might be improved.

Tendency for higher CR was found in animals fed concentrates as a supplement to roughages than in those not fed concentrates. The importance of nutrition is also evidenced by the finding that cows with good BCS had a tendency to show higher CR than those with poor BCS. Thus improved nutrition will not only reduce the postpartum anoestrous period as shown in many studies, but also improve CR. While recognising the possibility of direct effect of nutrition on fertilisation and subsequent events, the finding that farms with higher proportions of their total family income from dairying had shown tendency for higher CR than those with lower proportions, nevertheless indicate that these families attached more importance, and hence paid more attention, to the care of their cows. Presumably, this may include better feeding and heat detection, getting the cow served at the right time with respect to oestrus, resulting in higher fertility.

It is well known that the timing of insemination relative to first detection of heat is of a critical nature for achieving high CR [15]. In the present study, a trend in CR was shown with time from detection of oestrus to service; the CR increased as the interval increased from 6 up to 24 hours, and then declined. In theory, the optimum time for service is 12–18 hours after onset of "standing" heat. However, in the present study, the animals were housed or tethered and there was no opportunity for them to exhibit standing heat. Thus, it is likely that the farmer’s reliance on signs such as vaginal mucous discharge and bellowing may have resulted in animals being detected during pro-oestrus, several hours before the actual onset of oestrus. This could explain the above results, since AI done 18–24 hours after detection by the farmer would mean 12–18 hours after onset of standing heat. Incorrect timing of AI can be a major constraint in many regions of Sri Lanka. This stems from the ignorance of farmers regarding the importance of correct timing of service and the poor communication between smallholdings which are scattered and the AI service centres which are few in number. Attention therefore needs to be focused on addressing these deficiencies.

Variations in fertility due to bulls were also observed. Of the seven bulls from which semen had been used for at least 20 inseminations, one had very poor CR (18.2%), while two others had CRs below 45%. The continued use of such bulls in an AI programme is clearly unwarranted. A regular programme should therefore be instituted to monitor each bull used in AI and to cull those that have low fertility. Semen produced locally gave higher CR than imported semen. Assuming that the imported semen was of good quality at the point of origin, problems during subsequent transport, storage and/or handling could be responsible for the decline in fertility. Although the number of observations was low, a tendency for higher CR with chilled than with frozen semen was seen. This further stresses the need for special care in all operations associated with frozen semen. The findings of this study emphasise the need for provision of optimal conditions for transport and storage of semen, and also for routine monitoring of quality at the point of receipt and during storage.

The influence of the technician on the outcome of AI is well documented. Not only their skill, but also motivation, attitudes and the facilities available have profound influence on the outcome of AI. In the present study, only one technician was monitored in each of three veterinary ranges, while two were monitored in the fourth range (Yatinuwara). Thus location was a confounding factor in interpreting the influence of technicians on CR in this study. However, the wide range of CR seen between individual technicians (27.8–58.5%) is noteworthy. Also, both technicians monitored at Yatinuwara (the location which recorded lowest FSCR and OCR) had the lowest CRs. Although further studies are needed to partition the effects of factors such as location, bull, semen type and technician in order to evaluate the true effects of technicians on CR, it is very conceivable that technicians may have contributed substantially to the variation in CRs in the present study.

4.3. Large farms

Of the four farms studied only one has achieved CRs at least in the lower level of the acceptable range, while the other farms recorded even lower rates. This is a matter for concern as these farms are the premier state farms for multiplication of *Bos taurus* cattle in the country and are kept for the purpose of providing superior breeding animals to the smallholder farmers.

A recent study has shown that less than 75% of total potential land extent on these farms have improved pasture and the stocking rate is much lower than the potential [12]. In one farm, the body weights of the mature animals are much lower than those expected for that genotype. This may be most likely due to sub-optimal nutrition and lack of a regular selection and culling policy for desired breed characteristics and performance. The sub-optimal feeding of animals was reflected in BCS, with most animals recorded at less than 2.5. In general all four farms do not appear to practice a regular systematic culling programme on the basis of age, productivity and reproductive performances as reflected in the heterogeneity of the cattle population in terms of parity. As shown in the present study, the older animals tend to be less fertile. Therefore, sound management coupled with regular culling is of paramount importance to achieve higher fertility in these multiplier farms.

The average CFSI in all farms were well over three months. This delay may be due to long periods of postpartum anoestrus and, judging from the body condition of these animals, this is most likely caused by poor nutrition, particularly during the early postpartum period. Though the animals on New Zealand farm had relatively better body condition, yet their production levels were higher than those of animals at other farms. Presumably, these high yielding animals may have run into nutritional limitations particularly during the immediate post-calving period because of a greater drain of nutrients through milk. With overall average of two services per conception, the overall CCI was well over 5 months. At this level of efficiency of postpartum fertility, the average inter-calving interval was estimated to be 18 months, which cannot be considered to be satisfactory for a multiplier farm.

Although the average milk production was superior at New Zealand farm, the indices of fertility were inferior to those in the other three farms, where these were in any case lower than the desired level. Many factors may have contributed to this situation: firstly, sub-optimal nutritional management; secondly, a high proportion of older animals; and thirdly, as discussed later, poor heat detection.

As evidenced from progesterone concentration in milk samples, the accuracy of heat detection and the timing of insemination at all farms was less than optimal. Based on progesterone levels on day of AI (day 0), 10% of the animals could not have been in oestrus. When progesterone levels in the two samples on days 0 and 10–12 were considered, only 67% appeared to have had a normal ovulatory oestrus. If the samples with inconclusive progesterone data are also included, 36% of the cows presented for AI were not correct candidates to receive an AI; they were either anoestrous, pregnant or had an abnormal oestrous cycles. Thus oestrous detection efficiency appears to be a major factor contributing to the low success rate of AI. This point is supported by the finding that the farms which coupled visual heat detection with teaser bulls had higher CRs compared to those farms that relied only on visual heat detection.

The timing of AI relative to first detection of heat is known to be critical for achieving high CRs [15]. In theory, higher conception rates can be achieved if animals are inseminated between 12–18 hrs after detection of heat. In the present study AIs were performed at time intervals ranging from 1 to 24 hours and, when grouped into 3 time intervals (0–6, 6–12, and 12–24), the respective CRs were 60, 49 and 51%. This pattern of conception in relation to timing of AI could have been most likely due to the herdsmen’s inability to differentiate prooestrus and oestrus, and to make a correct judgement of the time of onset of oestrus.

Another factor which appears to have a significant effect on CR following AI is semen quality. It is apparent that factors relating to the chain of activities in performing AI such as transport, storage and handling of semen during thawing and the AI technique itself are very important determinants of CR [15]. Findings of on-going research in our laboratory indicate that there is significant reduction in quality of semen during storage and transport. There was a drop of sperm motility from 55 to 50% upon dispatch from the point of production to point of use. At the point of use there was again a significant reduction of motility with time, dropping to less than 30% over a 3 month storage period. As we have not looked into this aspect in the present study, conclusions cannot be made on the possible contribution of semen quality to recorded conception rates. With regard to technique of insemination, there appear to be differences between technicians, which may be related to skill, motivation, attitudes and facilities available to them. A wide difference was seen in CRs achieved by individual technicians. The lowest fertility was seen at the farm which was served by the technician with lowest achievement in CR. Whether this is a reflection of the technician's poor performances or the result of sub-optimal management factors related to heat detection, timing of insemination or semen factors cannot be determined for certainty at this point. Of the 5 bulls from which semen was used in at least 20 inseminations, one had very poor CR (34%). It is clear that there is an influence of the semen donor in the observed variation in fertility. Further studies are in progress to partition the effects of factors such as heat detection efficiency, timing of insemination, bull and semen type, and technician in order to evaluate the true effects of these factors on the observed fertility status of artificially bred cattle in Sri Lanka.

5. CONCLUSIONS

In conclusion, the findings of the first study indicate that the proportion of breedable cattle served by AI and the overall success rate of AI are too low to make a measurable impact on the genetic composition of the national cattle population. Many factors have contributed to this low level of performance and efficiency, including farmer’s ignorance and low motivation, poor infrastructural facilities, inadequate veterinary coverage and resources, low motivation and mobility of field staff. The findings of the second study indicate that the overall success rate of AI in small-holder farms in the mid-country wet zone of Sri Lanka is in the lower range of acceptability, with high variability in CR between locations. Findings of the third study indicate that the overall success rate of AI in the four state multiplier farms studied was also lower than optimal. Both smallholder and large farm studies suggest that many factors appear to contribute to this low level of efficiency. Of these, poor heat detection and delays in getting the AI done stand out as the most important contributory factors. Results also indicate that the quality of semen, technical efficiency in its handling and storage, and the skill of AI technicians also contribute to this low success rate.

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CONSTRAINTS ON EFFICIENCY OF ARTIFICIAL INSEMINATION AND EFFECT OF NUTRITION ON REPRODUCTIVE PERFORMANCE OF DAIRY CATTLE IN SMALL HOLDER FARMS IN VIET NAM

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Abstract

CONSTRAINTS ON EFFICIENCY OF ARTIFICIAL INSEMINATION AND EFFECT OF NUTRITION ON REPRODUCTIVE PERFORMANCE OF DAIRY CATTLE IN SMALL HOLDER FARMS IN VIET NAM.

This report is the result of a survey on 564 cows subjected to artificial insemination (AI) from March 1995 to March 1996 in 4 districts around Ho Chi Minh City. Four inseminators filled in questionnaires relating to farms, semen batches, cows and inseminations done. Milk samples were collected and analysed for progesterone by radioimmunoassay (RIA). All raw data were stored and analysed by the computer program AIDA (Artificial Insemination Database Application). Conception rate at first service (FSCR) was 61.7% and overall conception rate (OCR) was 68.4%. The intervals from calving to first service (CSI) and calving to conception (CCI) were 108 days 119 days respectively. Cows with lower percentage of Holstein-Friesian (HF) blood had shorter CSI and CCI than those with higher percentage of HF blood. Other factors which influenced OCR, CSI and CCI were parity, high milk yield (>20 L/cow/day), inappropriate heat signs, slight degree of uterine tone, feeding without green grass, too early or too late intervals from heat signs to AI, time of AI and difficulty in passage of AI pipette. Progesterone measurement in 796 sets of milk samples indicated that AI in 546 cows (68.6%) resulted in conception and they were subsequently diagnosed pregnant, while 24 animals (3%) conceived but underwent late embryo mortality. In 146 cows (18.3%) AI was done in the follicular phase but conception did not occur. There were 73 cows (9.1%) where progesterone values were intermediate (1–3 nmol/L). Ninety-four Holstein crossbred cows (F1 and F2) were assigned into three groups by different metabolisable energy (ME) ratios between demand and supply in order to determine effect of nutritive value of rations on reproduction. The cows in ME-balanced group were also divided in two sub-groups by different quantity of green grass in the ration to evaluate influence of the latter on fertility. Results showed that only cows fed with balanced rations in ME achieved good fertility (calving to first heat 59 days, CSI 74 days, CCI 96 days and services/conception 1.63). Moreover, when the feed fully provided ME and green grass (≥20 kg/head/day) reproductive performance increased considerably (calving to first heat reduced to 50 days, CSI to 64 days, CCI to 82 days and services/conception to 1.4).

1. INTRODUCTION

The dairy cow industry is developing rapidly in areas surrounding Ho Chi Minh City, especially in some districts such as Hoc Mon, Go Vap, Tan Binh, Thu Duc. The number of dairy cows in this area is about 19 840 heads. These districts comprise 79% of dairy herd in South Vietnam and 65% of dairy herd in whole country.

Dairy herds will continue to develop as the main objective of Ministry of Agriculture and Rural Development is to reach 41 000 heads of dairy cows in the year 2000. So, it needs to combine areas such as improvement of breed of dairy cow and increasing performance. At present, fertility of dairy cows is not efficient, with long calving intervals from 13.6–16.5 months [1, 2]. Therefore, improvement of reproduction is very important to reach the necessary population of dairy cows in the future.

Although artificial insemination (AI) for dairy cows started many years ago, constraints on the efficiency of AI have not been fully understood. So, a survey is needed to assess the present situation of AI and to find out the factors affecting it.

It is estimated that 80% of the variance in fertility is due to environmental factors, of which more than 50% is explained by nutrition [3]. While feeding management for dairy cows have not been improved by farmers in smallholder farm; fertility of dairy cows will be affected considerably. Eighty eight percent of rations for dairy cows in surrounding Ho Chi Minh City are not adequately balanced.

It has been shown that the reproduction of dairy cows will increase after their nutritive requirement has been provided fully by balance rations. However, there have been no trials to study the effect of nutrition on fertility of dairy cows in South Vietnam. Most of the trials have been concentrated to find out the influence of nutrition on milk yield. So, this trial was done to assess the role of nutrition on reproductive performance in dairy cows raised under the conditions of South Vietnam.

2. MATERIALS AND METHODS

2.1. General information on survey area

Total number of dairy cows in Ho Chi Minh City and surrounding area in 1998 was 19 840 heads. Dairy cows in the four districts included in the survey occupied about 80% of the total and they provided about 22 961 tons of fresh milk per year. There are two major seasons in this area. Rainy season is from May to October with average rainfall of 314 mm, air temperature 27.8°C and relative humidity 82%. Dry season is from November to April with monthly average rainfall of 33 mm, air temperature 27.2°C and relative humidity 73%.

2.2. Baseline survey

This were undertaken from March 1995 to March 1996 on 564 inseminated Holstein-Friesian (HF) crossbred cows in 480 smallholder farms in four districts of Ho Chi Minh City (GoVap, Tan Binh, Thu Duc, Hoc Mon district). Four trained inseminators were included, one for each district. They filled in the questionnaires which had been designed by IAEA and translated by the Institute of Agricultural Science. Three samples of milk were collected for each AI: on the insemination day (day 0), 10 days later and 21–24 days after AI. Rectal palpation was done at 75–90 days after AI in order to determine pregnancy. Quality of semen (concentration and activity) was evaluated by using a light-microscope. The AIDA software (Artificial Insemination Database Application, IAEA, Vienna) was used to record and to analyse the data.

All data were classified under five main groups which may influence efficiency of AI as follows:

- Cow factors including two sub-factors: reproductive characteristics of cow (breed, parity, calving state, milk yield, milking state) and heat signs of cow;
- Farm factors: management system and knowledge of farmers on feeding and other aspects;
- Semen factors: source of semen, type of semen, bull breed, activity of sperms after thawing;
- Inseminator factors: skill and experience of inseminators;
- Season factor: dry and rainy season.

Conception rate (CR) was calculated for each group. Chi-square test and T-test were used to compare the CR, interval from calving to first service (CSI) and interval from calving to conception (CCI) between the groups.

2.3. Interventions

After having the results of the survey an intervention aimed at reducing the intervals from calving to progesterone elevation, heat, AI and conception was undertaken using different rations. The trial was conducted from June 1997 to April 1998 on 94 heads of F1 and F2 HF crossbred cows in four districts in surrounding Ho Chi Minh city, including: Go Vap, Tan Binh, Hoc Mon and Thu Duc district.

The cows were divided in 3 groups based on a difference in Metabolisable Energy (ME) between demand and supply, as follows:

- ME-Deficient: the difference between demand and supply was less than –10% (n = 25). The major part of diets included natural grass, concentrate and some agro-industrial by-products (AIBP) such as beer, cassava and soybean residues. The diet provided about 12.4 Mcal of ME, 0.8 kg of crude protein (CP) and 5.3 kg of dry mater (DM) in dry period and 21.2 Mcal of ME, 1.4 kg of CP, 9.1 kg of DM in lactating period.

- ME-Balanced: the difference between demand and supply was ±10% (n = 43). The diets included: natural grass, concentrate, beer residue, cassava residue, soybean residue and rice straw. Diet provided about 16.6 Mcal of ME, 0.9 kg of CP and 7.7 kg of DM in dry period and 26.7 Mcal of ME, 1.6 kg of CP, 11.9 kg of DM in lactating period.
- ME-Excess: the excess ME supply over demand was more than +10% (n = 26). The diets included natural grass, concentrate and residues of beer, cassava and soybean together with rice straw. The diet provided about 19.1 Mcal of ME, 1.2 kg of CP and 9.1 kg of DM in dry period and 29.8 Mcal of ME, 1.8 kg of CP, 14.5 kg of DM in the lactating period.

Quantity of each component of ration was weighed and recorded on a notebook computer by technicians, twice a month. Further, the nutritive value of ration (ME and CP) was evaluated by using results available in the Institute for Agricultural Science from previous analyses of major feedstuffs available for dairy cows in the areas surrounding Ho Chi Minh City [4, 5]. Demand of dairy cows in different stage (dry, lactating or pregnant period) in ME and CP was determined by using standards of the Nutrition Research Center [10]. Twice a month, body condition score (BCS) was estimated on a 1–5 scale and body weight (kg) was measured using a Weigh-Band (Dalton, UK).

Based on previous studies in dairy cows, 20 kg/head/day of grass in a ration is regarded as sufficient. So, dairy cows in the ME-Balanced group were divided into two sub-groups: grass-sufficient and grass-deficient. The animals were fed with two different diets. The first was a mixture of mainly natural grass, concentrate, residues of beer, cassava and soybean together with rice straw providing about 24.4 Mcal of ME, 1.4 kg of CP, 11.1 kg of DM and 18.5% of DM from forage in lactating period. The second was a mixture of mainly natural grass, concentrate, beer residue, cassava residue and rice straw providing about 28.7 Mcal of ME, 1.7 kg of CP, 12.6 kg of DM and 38.9% of DM from forage in lactating period.

Milk samples were collected every 5 days from 30 days after calving until conception. Progesterone level in milk samples was determined by RIA technique using kits supplied by the IAEA, Vienna. Reproductive performance of cows was determined by calculating the interval from calving to progesterone elevation, CSI, CCI and number of services per conception (S/C). All raw data was recorded in Excel and transferred to SYSTAT for further analysis.

3. RESULTS

3.1. Baseline Survey

3.1.1. Efficiency of AI in surveyed-area

A total of 825 inseminations were recorded on 564 cows. The first service conception rate (FSCR) was 61.7%, the overall conception rate (OCR) was 68.4% and S/C were 1.5. Intervals from calving to first service and conception were rather long (108 and 119 days, respectively).

3.1.2. Effect of factors related to cows and heat signs on CR

As shown in Table I, CR was reduced progressively when the percentage of HF blood increased from 50% through 75% to 87.5%. However, the difference in CR between these groups was not significant ($P > 0.05$). Increase of HF blood also lengthened the CSI and CCI, and this was significant between groups ($P < 0.05$). The cows with higher parity (>2 and up to 5) had a better CR than those with lower parity. However, there was no significant difference in CSI and CCI between these groups ($P > 0.05$).

The fertility of cows with medium milk yield (11–20 L/cow/day) was higher than that of other groups. Cows with high milk yield (>20 L/cow/day) had low CR (57.1%) and high S/C (1.8), while cows with low milk yield (<10 L/cow/day) had highest CR (76%) and lowest S/C (1.3). However, the CSI and CCI of the latter were longer.

There was significant difference in CR between cows subjected to milking + suckling, compared with those subject to milking only (64.3% compared with 81.8%, P <0.001). However, the CSI and CCI of the former group were shorter than those of the latter group (P <0.01).

TABLE I. EFFECT OF COW FACTORS ON OVERALL CONCEPTION RATE (OCR), SERVICES PER CONCEPTION (S/C), CALVING TO FIRST SERVICE INTERVAL (CSI) AND CALVING TO CONCEPTION INTERVAL (CCI)

Parameters	No. of Services	No. of Conceptions	OCR (%)	S/C	CSI (days)	CCI (days)
Cow breed:	(a)					
50% HF crossbred	234	171	73.1	1.4	103 ± 32	112 ± 37
75% HF crossbred	549	368	67.0	1.5	110 ± 35	122 ± 40
87.5% HF crossbred	30	19	63.3	1.6	111 ± 32	125 ± 36
Parity:						
1	325	213	65.5	1.5	104 ± 37	117 ± 42
2	274	186	67.9	1.5	110 ± 35	121 ± 39
3	110	81	73.6	1.4	112 ± 27	121 ± 33
4	66	46	69.7	1.4	103 ± 34	115 ± 38
5	40	32	80.0	1.3	112 ± 36	119 ± 39
6	8	5	62.5	1.6	103 ± 18	116 ± 27
Milk production on day of service (litres/cow/day):						
≤ 10	25	19	76.0	1.3	129 ± 57	154 ± 66
11–15	305	219	71.8	1.4	111 ± 37	125 ± 41
16–20	455	301	66.2	1.5	104 ± 32	112 ± 34
>20	35	20	57.1	1.8	116 ± 28	125 ± 29
Lactation state:						
Milking + Suckling	628	404	64.3	1.6	106 ± 34	107 ± 32
Milking only	187	153	81.8	1.2	121 ± 43	150 ± 36

Note: (a) numbers within columns do not add to total number of services due to missing data

The influence of factors related to heat signs is given in Table II. Most cows were inseminated when having mucus and standing signs (63.4% and 29.2%, respectively). CR of these two groups was high (68.2% and 79.9%, respectively). CR of those inseminated when restless or mounting others was lower (23.3% and22.2% respectively). In 98% of the cases AI was done with clear mucus discharge and CR was 68.9%. Out of 8 cows inseminated with turbid mucus discharge only one became pregnant (CR 12.5%).

The majority of cows (83%) were inseminated with slight swelling of the vulva and they had a higher CR (70.4%) than those inseminated with a marked swelling of the vulva (61.2%, P <0.05). The interval from first heat signs to AI depends upon heat detection by farmer and the time that farmers inform the inseminators. CR was low when these intervals had been either early (<12 hours, 61.6%) or late (>42 hours, 27.3%). CR was best when the interval for AI service had been 18–24 hours. Surveyed results showed that 22.6% of cows were inseminated either too early or too late after heat detection. There was a significant difference in CR between cows inseminated in the morning (78.2%) and in the afternoon (56.6%, P <0.001).

TABLE II. EFFECT OF HEAT SIGNS ON OVERALL CONCEPTION RATE (OCR), AND SERVICES PER CONCEPTION (S/C)

Parameter	No. of services	No. of conceptions	OCR (%)	S/C
Heat signs observed by farmers:				
Standing	239	191	79.9	1.3
Mucus	519	354	68.2	1.5
Restless	43	10	23.3	4.3
Mounting others	18	4	22.2	4.5
Swelling of vulva:				
Marked	147	90	61.2	1.6
Slight	673	474	70.4	1.4
Interval from first heat sign to AI (h):				
12	88	53	61.6	1.7
18	471	349	74.1	1.4
24	172	109	63.4	1.6
30	33	20	60.6	1.7
36	47	25	53.2	1.9
>42	11	3	27.3	3.7
Time of AI:				
Morning	449	351	78.2	1.3
Afternoon	376	213	56.6	1.8

3.1.3. Effects of region and feeding system

The reproductive indices in the four districts and in farms with different feeding systems are shown in Table III. Feeding systems with green grass (supplement at shed) had higher CR than feeding system without green grass (65.6–72.7% compared with 55.6%). Systems without grass feeding had number of S/C and also longer CSI and CCI than those with grass feeding.

TABLE III. EFFECT OF REGION AND FEEDING SYSTEM ON OVERALL CONCEPTION RATE (OCR), SERVICES PER CONCEPTION (S/C), CALVING TO FIRST SERVICE INTERVAL (CSI) AND CALVING TO CONCEPTION INTERVAL (CCI)

Parameters	No. of Services	No. of Conceptions	OCR (%)	S/C	CSI (days)	CCI (days)
Region:						
Go Vap	115	83	72.2	1.4	114 ± 15	123 ± 18
Tan Binh	111	74	66.7	1.5	107 ± 28	119 ± 23
Thu Duc	21	14	66.7	1.5	106 ± 14	117 ± 20
Hoc Mon	574	389	67.8	1.5	106 ± 35	118 ± 38
Feeding system[1]:						
Grass + Conc. + AIBP	770	528	68.8	1.5	107 ± 35	119 ± 39
Grass + Conc.	32	21	65.6	1.5	97 ± 28	110 ± 33
Grass + AIBP	11	8	72.7	1.4	114 ± 28	125 ± 36
AIBP + Conc	9	5	55.6	1.8	144 ± 40	161 ± 29

[1] Conc. = Concentrate feed; AIBP = Agro-industrial by-products (including rice straw, cassava residue, soybean residue, sugar-cane tops and pineapple peel)

3.1.4. Effects of semen and inseminator

There was no significant effect of source of semen (imported or local), type of semen (pellet or straw) and breed of bull on CR (P >0.05). However, activity after thawing (Table IV) had a significant effect on CR (P <0.01).

Generally, there was no significant difference in CR between inseminators (P >0.05). However, when the degree of uterine tone was slight, manipulation of inseminator inside reproductive tract of cow has been difficult so CR decreased (25.9% with slight degree of uterine tone compared with 71.4% with marked degree of uterine tone, P <0.001).

TABLE IV. EFFECTS OF SEMEN QUALITY AND EASE OF AI ON CONCEPTION RATE

Parameter	No. of Services	No. of Conceptions	Conception Rate (%)	Services per Conception
Motility before AI:				
30%	206	142	68.9	1.5
40%	460	297	64.6	1.5
50%	153	121	79.1	1.3
Ease of passage of pipette:				
Difficult	54	14	25.9	3.8
Easy	770	550	71.4	1.4

3.1.6. Effect of season on CR

Although CR showed variations during different months, there was no significant difference in CR between rainy season and dry season (68.8% compared with 68%, P >0.05).

3.1.7. Interpretation of progesterone data and outcome of AI

Progesterone measurement in samples collected on day 0 showed that 98.5% of cows had been inseminated when progesterone was low (i.e. not in luteal phase). Based on three samples, 71.6% of animals appeared to have conceived (values were low, high and high respectively); however, 3% of these probably had late embryonic mortality. Only 0.4% of cows were inseminated while being pregnant and 0.5% of cows were inseminated in anoestrus. Eighteen percent of the cows which were inseminated in follicular phase did not conceive and a return to oestrus was not detected until manual PD. The results based on three samples an subsequent clinical pregnancy diagnosis are shown in Table V.

TABLE V. PROGESTERONE DATA FROM THREE SAMPLES AND SUBSEQUENT CLINICAL PREGNANCY DIAGNOSIS (PD) IN INSEMINATED COWS

Day 0	Day 10–12	Day 22–24	PD	No. of cow	%
Low (023 ± 0.21)	High (9.59 ± 3.98)	High (13.04 ± 5.92)	Positive	546	68.6
Low (0.45 ± 0.17)	High (7.50 ± 4.80)	Low (0.34 ± 0.25)	Negative	146	18.3
Low (0.29 ± 0.27)	High (9.39 ± 4.66)	High (8.15 ± 4.88)	Negative	24	3.0
High (4.90 ± 0.80)	High (14.36 ± 10.51)	High (13.20 ± 8.68)	Positive	3	0.4
Low	Low	Low	Negative	4	0.5
Intermediate*	Intermediate*	Intermediate*		73	1.1
		Total		796	100

* Intermediate value of progesterone (1–3 nmol/L)

3.2. Interventions

3.2.1. Effect of nutritive value of ration on fertility of dairy cows

3.2.1.1.Nutritive value of rations in two periods, pre and post calving

Results in Table VI show that the difference in ME between demand and supply for the dairy cows studied had not changed markedly between the pre and post-calving periods. In the ME-deficient group there were only 5 cows fed with rations having balanced or excess ME in the post calving period and they were eliminated from the analysis. Similarly, 4 cows in the ME-excess group were eliminated because they had been fed ME-deficient or ME-balanced rations during the post-calving period.

TABLE VI. NUTRITIVE VALUE OF RATIONS FED TO DAIRY COWS DURING PRE- AND POST-CALVING PERIODS

Parameter	Cow Group		
	ME-Deficient	ME-Balanced	ME-Excess
Number of cows	25	43	26
Pre-Calving Period:			
Demand – supply difference			
% ME	- 24.9 ± 10.8***	- 0.3 ± 5.1	+ 21.7 ± 7.3***
% CP	- 11.2 ± 21.7*	+ 3.4 ± 15.8	+ 43.7 ± 16.3***
Body score (1–5 scale)	2.8 ± 0.2***	3.3 ± 0.3	3.6 ± 0.2**
Body weight (kg)	456 ± 58	461 ± 69	434 ± 49
Post-calving Period:			
Demand – supply difference			
% ME	- 20.9 ± 7.6***	- 5.2 ± 2.6	+ 22.4 ± 6.2***
% CP	+ 2.3 ± 17.2	+ 7.5 ± 8.4	+ 54.4 ± 23.6***
Body score (1-5 scale)	2.7 ± 0.2***	3.2 ± 0.2	3.3 ± 0.1
Body weight (kg)	440 ± 55	443 ± 65	415 ± 43
Milk Yield (Litres/head/day)	13.3 ± 3.7	14.5 ± 3.9	11.7 ± 3.1

ME – metabolisable energy; CP – crude protein

Significant difference compared with ME-Balanced group: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

3.2.1.2.Nutritive state of dry cows:

Rations were calculated to have supplied about 16.2 Mcal ME and 0.9 kg CP to dry cows. Differences of ME and CP between ME-balanced group and the other two groups were significant (P <0.05). BCS accurately indicated the state of nutrition of dry cows. ME-deficient group had only reached BCS of 2.4–3.3 whereas ME-excess group had BCS of 3.0–3.8. Difference of BCS between ME- balanced group and other two groups was significant (P < 0.001).

3.2.1.3.Nutritive state of lactating cows:

Difference between demand and supply of ME was similar during then post-calving period as during the pre-calving period. BCS of cows decreased about 0.1–0.2 points in all three groups. Results presented in Table VI show that: the ME-deficient state is more common in dairy cows with milk yield greater than 13 L/head/day, whereas cows with milk yields lower than 12 L/head/day are usually in ME-excess.

3.2.1.4.Effect of nutritive value of ration on reproductive performance of cows

The results are summarised in Table VII. Intervals from calving to heat, AI and conception were significantly longer, CR was lower and incidence of retained placenta were higher in the ME-excess group when compared with the ME-balanced group. The ME-deficient group had the lowest

CR and highest rate of retained placenta. There was a significant difference in the interval from calving to first heat between the ME-deficient and ME-excess groups (P <0.05). Cows in the ME-balanced group had the highest CR (61.35%) and lowest incidence of retained placenta (2.3%).

TABLE VII. EFFECT OF NUTRITIVE VALUE OF RATION ON REPRODUCTION OF COWS

Parameter	Cow group		
	ME-Deficient	ME-Balanced	ME-Excess
Number of cows	25	43	26
Interval (days) from Calving to:			
Progesterone elevation	70 ± 27	60 ± 15	88 ± 27
First heat	81 ± 31	59 ± 23	97 ± 29***
First AI	105 ± 26**	74 ± 25	113 ± 25***
Conception	142 ± 22**	96 ± 31	142 ± 28**
Services/conception	2.20 ± 0.71*	1.63 ± 0.69	1.88 ± 0.71
Retained placenta (%)	24.0	2.3	11.5

ME – metabolisable energy

Significant difference compared with ME-Balanced group: * P < 0.05; ** P < 0.01; *** P < 0.001

3.2.2. Effect of quantity of green grass of ration on fertility of dairy cows

All groups had been fed with adequate rations but quantity of natural green grass was different. The results (Table VIII) show that although BCS between the groups was not significant (P >0.05), the intervals from calving to heat and AI for the group receiving sufficient green grass shorter, (P <0.05 and P <0.01) and CR was higher (71.9%, P <0.01).

Deficiency of green grass in rations of lactating cows influenced reproduction negatively. All reproductive indices of cows in grass-deficient group were greatly reduced when compared with the grass-sufficient group, although they had been provided enough quantity of energy.

TABLE VIII. EFFECT OF QUANTITY OF GREEN GRASS OF RATION ON REPRODUCTION OF DAIRY COWS IN POST-CALVING PERIOD

Parameter	Green grass in feed	
	Deficient (< 20kg/head/day)	Sufficient (≥ 20kg/head/day)
Number of cows	20	23
Difference between:		
ME Demand – Supply (%)	- 4.9 ± 2.9	- 5.3 ± 2.4
CP Demand – Supply (%)	+ 3.3 ± 0.3	+ 5.5 ± 3.8
Body condition score (1–5)	3.3 ± 0.2	3.2 ± 0.1
Body weight (kg)	415 ± 68 *	468 ± 52
Milk yield (L/head/day)	13.0 ± 4.3 **	15.8 ± 3.2
Interval (d) from calving to:		
First Heat	70 ± 26*	50 ± 17
First AI	85 ± 26*	64 ± 21
Conception	112 ± 36**	82 ± 16
Services/conception	1.9 ± 0.8**	1.4 ± 0.5

ME – metabolisable energy; CP – crude protein

Significant difference compared with ME-Balanced group: * P < 0.05; ** P < 0.01; *** P < 0.001

3.2.3. Relationship between body condition with milk yield and nutrition

The body condition of lactating cows showed a sharp decline in early lactation, up to the third or fourth month after calving, and then increased from the fifth month of lactation period. It appears that body condition was inversely proportional to milk production and ME difference. The loss of body condition after calving in cows in the ME-Deficient group was less than that in the ME-Balanced and ME-Excess groups. This is likely to be due to the lower body condition score of this group at at calving and their lower milk yield compared to the other two groups.

4. DISCUSSION

4.1. Basic survey

The results obtained in the baseline survey on the conception rate to AI were acceptable and similar to previous results in countries which have good dairy production systems [6, 7]. However, the intervals from calving to heat, first AI and conception were longer than the optimum periods considered to be economically desirable. The level of milk production on insemination day had a significant influence and was probably caused by inadequate feed given to the cows with high milk yields. It was also evident that feeding systems which included adequate green grass in the ration resulted in higher reproductive performance of dairy cows.

All regions surveyed showed that farmers had good technical knowledge on dairy cow management, due mainly to the training courses which had been organized on a large scale and reaching to each village as part of the extension activities undertaken by the Institute for Agricultural Science. The significantly lower reproductive performance in one district (Go Vap) could be due to deficiency of green grass in the ration and the poor state of grazing because of greater urbanization.

The most suitable interval for AI is considered to be 18–24 hours after onset of standing heat signs [8, 9]. The results showed that 22.6% of dairy cows were inseminated either too early or too late in relation to heat detection. So, it is necessary to instruct farmers in the reliable signs of onset of heat and the best interval from first heat signs to AI.

The type of mucous discharge most suitable for conception was found to be a clear discharge, indicating that this signifies a normal situation of the cow’s reproductive tract. The effect of time of AI (AM better than PM) found in this study could reflect the effect of lower ambient temperature during the morning than in the afternoon, which is more conducive to conception [10].

4.2. Intervention study

4.2.1. Effect of nutritive value of ration on reproduction of dairy cow

Previous results [11] have shown that dry cows are usually fed with inadequate or unbalanced rations (about 16.2 MCal ME and 0.98 kg CP). In the pre-calving period the ME of rations have not met the requirements of cows in the ME-Deficient group. This could have been caused by deficiencies in natural grass and rice straw. In the post partum period rations were found to fully supply the requirements of CP to the cow. This was a result of farmers using more feedstuffs with high CP, such as: mixed bran, beer residue and soybean residue. However, farmers only used these feedstuffs in higher quantity for lactating cows.

The results showed that ME deficiency usually occurred in cows with milk yield higher than 13 L/head/day, whereas ME excess occurred in cows with yields less than 12 L/head/day. This was in agreement with previous studies in Vietnam [10, 11, 12]. Both deficiency and excess of ME have affected considerably the reproduction of cows in the present study. Energy is considered an important nutrient for dairy cows both before and after calving. Excess or deficiency of ME can lead to liver damage and indirectly influence reproduction, causing conditions such as ovarian dysfunction, silent heat, delayed ovulation and follicular cysts. Consequences of this problem have been reflected as long intervals from calving to first heat, AI and conception. Significant difference in these indices between the groups with ME deficiency and excess, compared with the group with balanced ME, was a clear demonstration of the effects of nutrition on reproduction. Previous studies have shown that an excess of ME causes delayed uterine involution and ovarian dysfunction through fatty liver syndrome [13, 14, 15]. There is a consensus among these authors that overfeeding should be avoided in the last stages of lactation to prevent fattening.

An energy deficiency before calving can lead to a higher incidence of retained placenta, endometriosis and low conception rates in the following lactation through ketosis and liver damage. On the other hand, energy after parturition is necessary for the onset of ovarian activity [16, 17, 18] and is related to involution of the uterus. The negative effect of an insufficient energy provision before calving will be enhanced by an energy deficiency after the following parturition [13, 19]. From the above it is clear that the worst fertility will be found in cows that are energy deficient.

In the present study, cows which were fed with a balanced ration in both periods, dry and lactation, had good reproduction. The intervals from calving to heat, AI and conception in this group were better than in the other groups, and also better than previous reports from a study in the same area in 1995 [12]. Balanced rations have affected positively not only reproduction, but also milk production. Therefore, balanced rations not only improve fertility of dairy cows but also directly increase income to the farmers. However, application of balanced rations for dairy cows is still unfamiliar to farmers because of reasons such as lack of knowledge and expertise in using the locally available feed resources such as agro-industrial by-products to effectively formulate the daily ration.

4.2.2. Effect of green grass on reproduction of dairy cow

There was clearly a beneficial effect of combining a balanced ration with enough quantity of green grass. Fertility of these cows was improved considerably by that combination. The economical benefits to the farmer were highest in the group with sufficient grass. However, the main constraint is that natural pastures have been reduced gradually due to urbanization and so farmers find it difficult to provide enough green grass for their dairy cows. Thus in some districts, particularly during the dry season, grass not available even though farmers are ready to buy it.

One way in which green grass of rations can affect indirectly the fertility of dairy cows is through its β-Carotene [20]. The β-Carotene in green grass is converted to Vitamin A in the ruminant's intestine and liver and the effect of Vitamin A on fertility has been clearly demonstrated [21]. However, there was not an absolute agreement between all authors on effect of Vitamin A and β-Carotene on fertility of dairy cows [20, 21, 22]. On the other hand, Vitamin E and phyto-hormones (such as those with estrogenic activity) in green grass can influence positively on reproduction of cows. Thus a common thumb-rule for forage feeding of lactating cow is that it should be kept to a minimum of 40% of the total dry matter of the ration [23].

4.2.3 Relationship between BCS and milk yield

Reduction of body condition in early lactation was caused by both negative energy balance and high milk production. Too thin cows at calving are lacking sufficient amounts of body reserves, which results in milk production increasing slowly after calving and the peak production being lower than expected. Thin cows come in heat later than those in good condition [24].

5. CONCLUSIONS

Reproductive performance of dairy cows has not yet been exploited fully under the conditions of management in the area of study, due to the long intervals from calving to first AI and to conception. Although the average CR was acceptable, it was low in cows which had parity <2 or >6, high milk yield on AI day (>20 L/cow/day), short or long CFAI (<60 days or >150 days), and abnormal heat signs (restless or mounting others). High percentage of HF blood (≥87.5%) only affected interval from calving to first AI and to conception. CR was low in farms having feeding system without (or deficiency of) green grass in the ration, in farms where interval from detection of heat signs to AI was too short (<12 h) or too long (>30 h), and where AI was done in the morning. Profile of progesterone during the post-partum period was a useful tool in order to evaluate the state of ovarian activity and to determine the correct treatment for disorders. In order to achieve good reproductive performance dairy cows need a balanced ration which meets their demand for energy and protein, together with sufficient quantity of green grass.

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STUDIES ON THE CAUSES OF INEFFICIENCY IN ARTIFICIAL INSEMINATION SYSTEMS IN DAIRY CATTLE IN ARGENTINA

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Abstract

STUDIES ON THE CAUSES OF INEFFICIENCY IN ARTIFICIAL INSEMINATION SYSTEMS IN DAIRY CATTLE IN ARGENTINA.

Information was obtained on reproductive efficiency and management from 17 dairy farms which use artificial insemination (AI) in Argentina. The methods included use of questionnaires, body condition scoring at calving and AI, measurement of progesterone in milk samples on day of AI (day 0), day 10–12 and day 22–24 by radioimmunoassay, and measurement of milk urea on day 0. The overall conception rate (OCR) from 504 inseminations was 41.5%. There was non-fertilization or early embryo death in 27.8% of cases and late embryo death in 10.4%. Three percent of inseminations were performed on pregnant animals, 8.2% with intermediate progesterone values (1.25–3.18 nmol/L) and more than 2% during anoestrus or with ovarian cysts. Calving season did not affect OCR, but first service conception rate (FSCR) was higher for cows calved during winter and bred in spring. Cows fed pastures and hay (P+H) had lower body condition at the time of AI than those fed pastures plus concentrates with or without hay. The P+H group showed delayed interval to first AI and conception, but higher FSCR. Both groups fed concentrates had higher milk urea that was related to lower OCR. Mean milk urea on the day of AI for cows that conceived was 43.81 ± 1.42 mg/100 mL, statistically different from the 48.87 ± 1.58 mg/100mL for those that did not conceive ($P < 0.05$). Inseminators formally trained for more than a month, employed on government farms and paid fixed salaries had better performances than those trained less than a month, employed on commercial farms and paid on the basis of inseminations or conceptions, respectively. OCR was also higher when cows were inseminated into the uterus, when uterine tone was slight, and mucus was clear, compared with those inseminated in the cervix, with marked uterine tone and without the presence of mucus, respectively. Semen of known good quality resulted in higher OCR than semen which was unexamined or without good information on its quality. However, 12 pregnancies resulted from 15 AIs done with semen classified as not good quality. This calls into question the criteria used for the classification of semen quality.

1. INTRODUCTION

In Argentina, nearly 70% of milk production is from three provinces: Santa Fé (26%), Cordoba (26.5%) and Buenos Aires (17%) [1]. Economic pressures, including the need to compete for new international markets require improved productivity of milk and hence application of technologies related to nutrition and artificial insemination (AI). Available technology can improve production to an average of 300 kg butter fat/ha/year, but of the three provinces Santa Fé produces approximately 115 kg/ha/year, and the other two between 60 and 90 kg/ha/year [1, 2].

Detailed national statistics on the usage of AI are not available. In Buenos Aires between 48 and 30% of the farms employ AI as a breeding tool. These are farms with a higher general level of technological development in nutrition, management and facilities. Reproductive efficiency is frequently less than optimal, with inter-calving intervals of greater than 13–14 months, 2 or more services per conception and 40–50% conception rates (CRs). Little is known about the reproductive wastage that occurs at different stages in the reproductive process or the amount of inefficiency that occurs at each step in the application of AI. Definition of this wastage and its economic significance is needed for planning for improvements in reproductive efficiency in the industry.

The objectives of this study were to: (a) use radioimmunoassay (RIA) of milk progesterone as a diagnostic tool to establish some parameters regarding reproductive efficiency; (b) use body condition scoring and milk urea as a way to assess nutritional status and investigate its possible contribution to reproductive failure; (c) study the influence of calving and insemination season, type of feeding, cow factors including milk production, heat symptoms, and semen quality on conception failure; and (d) determine which are the most common human factors causing poor reproductive performance including education level, income, work related factors and AI technique.

2. MATERIALS AND METHODS

2.1. Sites

The work was carried out on 4 farms in the province of Santa Fé, 4 farms in the province of Entre Ríos and 9 farms in Buenos Aires, from 1995 to 1998. The farms were selected based on farmers' willingness and ability to collaborate in the work. They were all in the area of the Humid Pampa that is characterized by mean maximum temperatures of 24–26ºC in January, and mean minimum temperatures of 10–12°C in July. There are 260–280 frost-free days/year. Rainfall is between 800 and 1000 mm/year and total solar radiation between 140–150 Kcal/cm^2/year, facilitating rapid growth of pastures. Argentinean Holstein was the predominant breed in the farms studied.

2.2. Records and measurements

Milk samples collected on the day of AI (day 0), on days 10–12 and days 22–24 were assayed for progesterone by the FAO/IAEA solid phase RIA [3]. Skimmed milk samples were frozen until assayed. Intra- and inter-assay coefficients of variation for quality control samples were 1.85% and 1.6% for a high pool and 4.3% and 8.3% for a low pool, respectively. Low and high progesterone concentrations were defined as lower than 1.25 nmol/L and higher than 3.18 nmol/L respectively. Intermediate values (between 1.25 and 3.18 nmol/L) were re-confirmed by repeating the analysis. Urea concentration was measured in frozen skimmed milk samples collected at the time of AI, after deproteinization with equal volumes of $Ba(OH)_2$ 0.3N and $ZnSO_4$, by the urease method (Lab. Wiener, Argentina) [4].

The inseminators recorded heat signs (standing, mounting, restless, or mucus), time of insemination (AM or PM) and some traits related with insemination (vulva swelling: marked, slight or none; presence of mucus: clear or none; uterine tone: marked, slight or none; pipette passing: easy, difficult or impossible; site of semen deposition: uterus, cervix or vagina). Some inseminations were performed after oestrous synchronization with two doses of $PGF_{2\alpha}$ 11 days apart, or CIDR for 9 days with an estrogen injection on day 1 and $PGF_{2\alpha}$ on day 8. Pregnancy diagnosis (PD) was carried out by rectal palpation between 45 and 60 days after AI. The performance of inseminators was examined in relation to their education level, age, number of AI carried out per month, years of experience, type of employer and method of payment.

Body condition score (BCS) was determined on a 1–5 scale [5] each time the farm was visited, at calving and at AI. Individual milk production per day was taken from monthly official records. The types of feeding practiced on the farm were classified as "Pasture plus hay" (P+H), "Pasture plus concentrates" (P+C) and "Pastures plus hay and concentrates" (P+H+C).

For the purposes of this project, AI Center personnel or private advisers classified semen batches after thawing, as being of good or not good quality based on percentage individual motility and vigour on a subjective 1–5 scale [6]. Where semen was not examined it was defined as "unexamined, probably of good quality" when it came from reputable internationally recognized centers or alternatively as having "no good information on quality".

Intervals from calving to first service (CSI) and calving to conception (CCI) as well as first service conception rate (FSCR) and overall conception rates (OCR) were calculated from farm reproductive records using the FAO/IAEA Artificial Insemination Database Application (AIDA). From a wider survey of farm size, farming enterprises and management practices, the following characteristics were selected for analysis of their relationship with farm conception rate: time spent on dairying, proportion of family income derived from dairying, season of calving and season of insemination.

Not all information could be collected on all farms. The number of farms and cows contributing to the different parameters is indicated in the tabulated results. Not all farms were visited during the same number of months. To avoid the influence of each farm on a given calving or insemination month, data were grouped by season.

2.3. Statistical analysis

Statistical analyses were done using Systat 6.01 for Windows [7]. Stem and leaf plots demonstrated that reproductive intervals did not follow a normal distribution. Cow age and parity were also skewed. Differences in the distributions of reproductive intervals between groups were compared with the Kruskal Wallis Test [8]. ANOVA was used to compare means of normally distributed continuous variables, like metabolic indicators (BCS and Milk Urea) [9]. The strength of association between independent continuous variables was tested by Pearson correlations [9].

Discrete variables such as conception were analyzed by chi-square when tested against other discrete independent variables [9]. For relationships between reproductive intervals and continuous variables linear regression was used. The level of significance was fixed at 95% for all the tests.

3. RESULTS

3.1. Survey of reproductive parameters using the radioimmunoassay of progesterone

Progesterone value on the day of AI was available for 460 inseminations and 92% of these were classified as low (<1.25 nmol/L), indicative of AI done at a time other than the luteal phase, including anoestrus, while 27% were classified as high (>3.18 nmol/L), indicative of AI done during the luteal phase (i.e. inappropriate). The progesterone value was intermediate in 11 cases (2%) and of these only 2 were subsequently diagnosed pregnant through rectal palpation.

Progesterone values from two milk samples were available for 82 inseminations (Table I). Eighty six percent of these inseminations were done with low progesterone values, from which nearly 77% were done during an ovulatory cycle.

TABLE I. DIAGNOSIS BASED ON PROGESTERONE IN SKIMMED MILK SAMPLES TAKEN FROM DAIRY COWS ON THE DAY OF AI AND DAY 10-12 AFTER AI

Day 0 (AI)	Day 10–12	Frequency (n)	%	Interpretation
Low	High	63	76.8	Ovulatory cycle
Low	Low	8	9.8	Anoestrus, anovulation or short luteal phase
High	High	2	2.4	AI on pregnant animal or luteal cyst
High	Low	0	0	AI during luteal phase
*	*	9	11.0	*At least one sample in intermediate range
		Total = 82		

For 327 inseminations all three milk samples were available for progesterone assay and PD was done by rectal palpation (Table II). Almost 47% of these inseminations were carried out when progesterone was low and were followed by evidence of cyclicity and pregnancy. Only 5 cows (2.6%) were diagnosed non-pregnant according to progesterone values on day 22 but were found pregnant through rectal palpation. Thus the accuracy of non-pregnancy diagnosis through milk progesterone in the present study was over 97%.

More than a quarter of the inseminations although carried out apparently at a proper time did not result in conceptions. Results suggest conception followed by embryo death after day 24 (or possibly the presence of a luteal cyst) in around 10% of cases. About 5% of AI were done while progesterone levels were high; rectal palpation indicated that pregnancy accounted for 3%, luteal phase for 1.5%, and luteal cysts for 0.9%.

Intermediate values were found in 17% fixed time inseminations after oestrus synchronisation. When cows were inseminated after visually detected heats this value varied between 3.6 and 7% in different farms. Percentage of late embryo mortality differed between farms from 3.6 to 23%. The latter occurred in one farm where none of the cows were confirmed as pregnant at time of PD.

TABLE II. DIAGNOSIS BASED ON PROGESTERONE IN SKIMMED MILK SAMPLES TAKEN FROM DAIRY COWS ON THE DAY OF AI, DAYS 10-12 AND DAYS 22-24 AFTER AI

Day 0 (AI)	Day 10-12	Day 22-24	Pregnancy Diagnosis	Frequency (N)	%	Interpretation
Low	High	High	Positive	153	46.8	Pregnant
Low	High	Low	Negative	91	27.8	Non-fertilisation, early embryonic mortality, post-AI anoestrus
Low	High	High	Negative	34	10.4	Late embryonic mortality (>day 16), luteal cyst, persistent CL.
Low	Low	Low	Negative	4	1.2	Anoestrus/Follicular Cyst
High	High	Low	Negative	5	1.5	AI performed during luteal phase
High	High	High	Positive	10	3.1	AI on pregnant animal
High	High	High	Negative	3	0.9	Luteal cyst, persistent CL.
*	*	*	Positive/ Negative	27	8.3	*At least one sample in intermediate range
			Total	327		

3.2. Survey of farm reproductive performance and management factors

3.2.1. Reproductive parameters

For the 17 dairy farms, median and inter-quartile range (IQR) for CSI and CCI were 77 (62 to 98) days and 87.5 (70 to 117) days respectively, but CSI ranged between 29 to 553 days and CCI between 39 and 541 days. Differences between farms were not significant except for one farm with extremely large intervals (CSI: 207 days, CCI: 221 days; $P < 0.05$). For 366 first services, conception rate was 41.6% and varied widely between farms from 0 to 68.8% ($P < 0.05$). Four of 17 farms had conception rates to first service over 50%. OCR for 504 services was 41.5% ranging from 0 to 70.6% ($P < 0.05$). Six farms had OCRs over 50%.

3.2.2. Season of calving

Calving season did not affect OCRs (47.6%, n = 82; 34.7%, n = 72; 37.5%, n = 195 and 47.4% in winter, spring, autumn and summer respectively), but FSCRs were higher for cows calved in winter (51.5%, n = 66) compared to those calved in autumn (36.8%, n = 125) and spring (34.0%, n = 47) (P = 0.05 and 0.06 respectively). FSCR for cows calved in summer did not differ from those in the other seasons (47.24%, n = 127). Table III shows the reproductive intervals for cows calved in different seasons.

TABLE III. INTERVALS FROM CALVING TO FIRST SERVICE (CSI) AND TO CONCEPTION (CCI) (MEDIAN AND INTER-QUARTILE RANGES) FOR COWS IN 17 ARGENTINEAN DAIRIES ACCORDING TO CALVING SEASON

CALVING SEASON	CSI (days)			CCI (days)		
	Median	IQR	(N)	Median	IQR	(N)
SUMMER	87.0^{a}	73.0–102.0	127	99.0^{ab}	78.0–117.0	91
AUTUMN	72.0^{b}	60.0–90.0	125	78.5^{a}	66.0–102.0	58
WINTER	69.0^{b}	55.7–86.0	66	75.0^{a}	50.0–89.0	39
SPRING	76.0^{ab}	61.0–121.0	47	139.0^{b}	61.5–204.5	25

IQR: Inter quartile range; Different superscripts within columns differ statistically ($P < 0.05$)

3.2.3. Season of insemination

Cows inseminated for the first time in spring had 57.1% FSCR (n = 56), higher than those inseminated in summer (30.8%, n = 26), autumn (43.9%, n = 157) or winter (37.0%, n = 127) (P <0.05). OCR was 50.0% (n = 74) in spring, higher than those in winter (38.9%, n = 167) and summer (23.5% n = 34) (P <0.05). Summer services had the lowest OCR (P <0.05). In autumn OCR was 46.3% (n = 229), not different from that in the other seasons, except summer.

3.3. Metabolic and productive parameters

BCS, urea and milk production for the 17 farms are shown in Table IV. The results indicate a significant variation between farms in all parameters (P <0.05).

TABLE IV. BODY CONDITION SCORES (BCS) AT CALVING AND AT THE TIME OF THE FIRST AI, MILK UREA (MU) ON THE DAY OF THE FIRST AI AND MILK PRODUCTION IN THE MONTH OF THE FIRST AI

	N	Mean	SD	Range in different farms (Mean ± SD)*
BCS at calving	80	3.46	0.49	2.7 ± 0.98 to 3.7 ± 0.22
BCS at first AI	276	2.57	0.59	1.9 ± 0.25 to 3.5 ± 0.25
MU on the first AI (mg/100mL)	266	46.54	17.7	31.8 ± 14.42 to 68.5 ± 4.80
Milk prod. in the month of first service (L/cow/day)	269	25.60	7.95	11.9 ± 3.96 to 36.3 ± 5.41

* All differences between farms were significant (P < 0.05)

3.3.1. Body condition

BCS at calving was lower in summer calving cows. Spring calving cows had the lowest BCS at the time of their first insemination (Table V).

TABLE V. BODY CONDITION SCORES (BCS) AT CALVING AND AT THE TIME OF THE FIRST AI GROUPED BY CALVING SEASON

SEASON	BCS at calving			BCS at first AI		
	LS Mean	SE	N	LS Mean	SE	N
AUTUMN	3.55[a]	0.09	22	2.60[a]	0.06	96
SPRING	3.61[a]	0.14	9	2.30[b]	0.08	45
SUMMER	2.75[b]	0.15	8	2.60[a]	0.05	152
WINTER	3.51[a]	0.06	41	2.79[a]	0.07	66

Within columns, different superscripts differ statistically (P < 0.05)

BCS at calving or at the moment of insemination did not influence FSCR or OCR at any season. BCS at calving was 3.50 ± 0.39 (mean ± SE, n = 30) for cows pregnant to first service and 3.48 ± 0.55, (n = 38) for cows that did not conceive to first service. Considering all services, BCS at insemination was 2.59 ± 0.59 (n = 178) for barren cows, and 2.60 ± 0.58 (n = 181) for the pregnant group. CSI was not correlated to BCS at calving (Pearson's Coef = -0.114, n = 68), but was significantly correlated to BCS at AI (Pearson's Coef = 0.123, n = 276, P <0.05).

3.3.2. Milk Urea

Milk urea at the time of insemination was lower in cows that conceived (43.81 ± 1.42 mg/100mL, n = 123) than in those that did not conceive (48.87 ± 1.58 mg/100mL, n = 153) (P <0.05). It was also more variable between seasons within barren cows than within pregnant cows (P <0.05) (Table VI).

TABLE VI. MEAN UREA CONCENTRATIONS IN MILK ACCORDING TO SEASON OF INSEMINATION AND SUBSEQUENT CONCEPTION

SEASON	Barren Cows			Pregnant Cows		
	LS Mean	SE	N	LS Mean	SE	N
AUTUMN	44.60[a]	1.73	92	41.65	1.53	94
SPRING	53.61 [b]	3.91	18	48.62	2.86	27
SUMMER	48.04 [b]	6.27	7	44.60	6.65	5
WINTER	53.70[b]	1.83	71	47.25	2.40	41

Within barren cows, different superscripts differ statistically ($P < 0.05$)

Within pregnant cows, differences between seasons were not significant. Within seasons, urea in barren cows was different from that of pregnant cows only in winter ($P <0.05$).

3.3.3. Metabolic indices in relation to types of feeding

Body condition and milk urea reflected nutrient supply in the different feeding types (Table VII). Compared to P+H+C, the group P+C had better body condition at calving, but this group was the one that had the lowest body condition at first breeding. Both groups receiving concentrates in their diets (with or without hay) had higher milk urea levels at the time of AI.

TABLE VII. BODY CONDITION SCORE (BCS) AT CALVING, AND BCS AND MILK UREA AT THE TIME OF THE FIRST AI IN COWS GROUPED ACCORDING TO TYPE OF FEEDING

Feeding type	BCS at calving			BCS at first AI			Milk Urea at first AI		
	LS Means	SE	N	LS Means	SE	N	LS Means	SE	N
Pasture + hay	--	--	--	2.55[ab]	0.10	30	31.76[a]	3.08	30
Pastures + concentrates	3.60[a]	0.07	38	2.45[a]	0.05	127	49.93[b]	1.49	127
Pastures + hay + concentrates	3.34[b]	0.08	30	2.71[b]	0.05	119	46.64[b]	1.61	109

Different superscripts differ statistically ($P <0.05$)

3.3.4. Type of feeding and reproductive indices

Cows fed P+H reached higher FSCR and OCRs (68.7%, n = 32 and 68.3%, n = 41) compared to those fed P + C (46.1%, n = 169 and 47.1%, n = 225) and P+H+C (33.5%, n = 165 and 34.4%, n = 238). Both indices were statistically different between all types of feeding ($P <0.05$).

The P+H group had longer CSI (median: 85.25 days, IQR: 96.5 to 107.5 days, =32) than the other two (respective medians, IQR and N: 77.5 days, 62.0 to 93.0 days, 168 and 75.0 days, 60.0 to 102.0 days, n = 165) ($P <0.05$).

The CCI from the P+H group (median: 99.5 days, IQR: 92.0 to 116.2 days, n = 28) was also higher than that from the P+C group (median: 84.0 days, IQR: 65 to 104 days, n = 105) ($P <0.05$), but the CCI from the group P+H+C (median: 84.5 days, IQR: 71 to 143.7 days, n = 80) was not different from the CCI of the other two.

3.4. Cow factors

Milk production did not affect OCR. Least square means for milk production (L/cow/day) ± SE was 25.56 ± 0.70 (n = 128) and 25.65 ± 0.67 (n = 141) for pregnant and barren cows respectively. It was negatively correlated with CSI (Pearson coef. = -0.34, n = 269, $P <0.05$) and CCI (Pearson coef. = -0.44, n = 167, $P <0.05$). Mean and inter-quartile ranges for cow age and parity were 6.78, 5.7–8.04 years and 2, 2–5 parturitions for pregnant cows and 7, 5.7–9.05 years and 3, 2–5 parturitions for barren cows. None of these differences was significant.

3.5. Effect of AI technician on CR

Technicians ranged in age from 19 to 63 years old with a mean of 40 years. From 21 inseminators, 15 had attended the primary school and 5 the secondary school. Only one had a professional degree. Their experience varied widely between 0 and more than 25 years (mean: 12.5 years). All of them had other duties also, in addition to AI.

Their performances were not related to education level, age or number of AI/month. OCRs tended to be higher in technicians between 11 and 20 years of experience (Table VIII). Technicians with fixed salaries obtained the best FSCR and OCR. Next to them came the owners or milkmen who were paid with a percentage of milk revenue and those who earned a basic salary plus bonus for conceptions. Technicians who worked for government research centers or agrarian schools had the better results on the first and all services as compared to private inseminators (Table VIII).

TABLE VIII. INFLUENCE OF TECHNICIAN ON FIRST SERVICE CONCEPTION RATE (FSCR) AND OVERALL CONCEPTION RATE (OCR)

	FSCR			OCR		
	(N)	%	p	(N)	%	P
Years of experience			0.28			0.06
<10	180	39.4		237	37.6	
11–20	34	52.9		46	54.4	
>21	152	44.7		221	46.2	
Method of payment			0.01			0.01
Fixed salary	129	51.9		174	54.6	
Basic + conceptions	122	32.8		169	36.7	
AI + conceptions*	15	0		23	0	
Owners-milk revenue	58	46.5		82	50	
Type of employment			0.13			0.01
Private	269	39.8		360	37.7	
Government	96	51.6		136	55.1	
Owners	4	50.0		8	62.5	
Formal training			0.02			0.01
< 1 month or none	250	38.4		342	38.0	
≥ 1 month	116	51.7		162	52.5	

* Data from only one technician

3.6. Proportion of family income derived from dairying

Table IX shows CRs from farms where different proportions of income were derived from dairying.

TABLE IX. FIRST SERVICE CONCEPTION RATE (FSCR) AND OVERALL CONCEPTION RATE (OCR) ON FARMS ACCORDING TO PERCENTAGE OF INCOME DERIVED FROM DAIRYING

Family Income (%)	Nº of farms	FSCR (%)	N	OCR (%)	N
0–25	2	37.5	12/32	35.55[a]	16/45
26–50	2	44.93	31/69	45.45[b]	40/88
51–75	4	50.52	48/95	54.28[b]	76/140
76–100	9	38.23	65/170	35.93[a]	83/231

FSCR: no significant differences; OCR: Different superscripts differ statistically, $P < 0.05$

3.7. Proportion of family time devoted to dairying

FSCR for different proportions of time devoted to dairying are shown in Table X.

TABLE X. FIRST SERVICE CONCEPTION RATE (FSCR) AND OVERALL CONCEPTION RATE (OCR) ACCORDING TO TIME DEDICATED TO DAIRYING

Family time (%)	Nº of farms	FSCR (%)	N	OCR (%)	N
26–50	2	44.9[a]	31/69	45.4[a]	40/88
51–75	4	51.4[a]	55/107	54.3[a]	82/151
76–100	11	36.8[b]	70/190	35.1[b]	93/265

Different superscripts differ statistically (P <0.05)

3.8. Factors related to heat and insemination

3.8.1. *Heat signs and AI techniques*

Conception rates in relation with heat signs and insemination procedures are given in Table XI. Seven cows were inseminated even though mucus was turbid or purulent. Only 2 of these became pregnant. These were not included in the statistical analysis because of the low number of observations. From 25 inseminations done at heats detected based on the principal secondary signs (mounting others, restless or presence of mucus), CR was 64 %. This was not statistically different from the CR of 47.1% for services done at heats detected based on standing behavior. The site of insemination was found to be important. When semen was delivered into the uterus insemination resulted in higher CR than when it was deposited into the cervix. Only 3 cows were inseminated into the vagina and none became pregnant (not included in the analysis). Conception rates after a synchronized heat were similar to inseminations after non-induced estrus. Differences were not significant between easy or difficult pipette passing, AM and PM inseminations or marked, slight or no vulval swelling.

TABLE XI. FIRST SERVICE CONCEPTION RATE (FSCR) AND OVERALL CONCEPTION RATE (OCR) ACCORDING TO HEAT SIGNS AND INSEMINATION FACTORS

	FSCR			OCR		
	(N)	%	p	(N)	%	p
Type of mucus			0.11			0.06
Clear	211	52.13		281	53.73	
None	50	46.00		77	41.56	
Uterine tone			0.05			0.007
Marked	100	41.00		133	40.00	
Slight	66	60.60		106	60.37	
None	19	47.30		21	52.40	
Semen site			0.48			0.003
Uterus	247	53.04		330	55.15	
Cervix	20	45.00		37	29.70	

CR was significantly lower when insemination was performed in the presence of marked uterine tone and when there was no mucus. Uterine tone increased as time elapsed from heat detection to insemination increased (Fig. 1). CR was not related to time elapsed since heat was first detected. CR was 54% for inseminations performed less than 12 hr from heat detection (n = 258) and 58% for AI done after a longer interval (n = 88).

3.8.2. Semen batch

Differences between CRs of cows grouped by semen type were significant (P <0.05, Table XII). CRs were higher when performed with semen from local bull stations (49.4%, n = 241) than when imported semen was used (37.05%, n = 197, P <0.05).

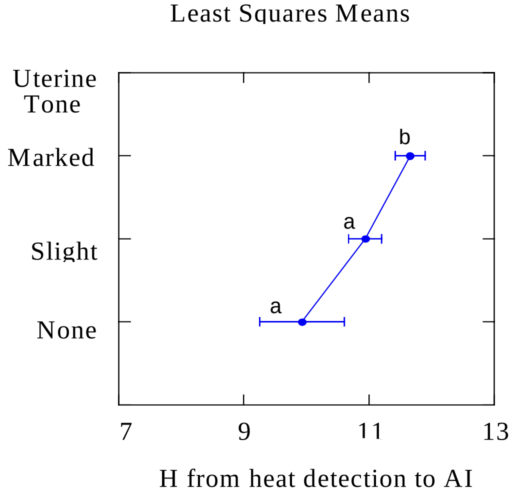

FIG. 1. Modification of uterine tone as time elapsed from heat detection to AI (a and b statistically differ; P <0.05, n = 276).

TABLE XII. RELATIONSHIP BETWEEN TYPE OF SEMEN AND CONCEPTION RATE (CR)

Semen Quality	Nº of services	Nº Conceptions	% CR
Good quality	190	95	50.0[a]
Unexamined, probably good quality	204	79	38.7[b]
No good information on quality	57	18	31.6[b]
Not good quality	15	12	80.0[c]

Different superscripts differ statistically (P <0.05)

4. DISCUSSION

The number of farms surveyed was small. The selection of farms on the basis of willingness and ability to co-operate in the studies would tend to bias the results towards farms where education and management competence are higher than average. In spite of this, the farms selected represent different farm sizes, feeding practices, production levels, reproductive performances and technician characteristics. Farms with the lowest technological level were not included because of the lack of records and due to difficulties in logistics of sample and data collection.

It was not possible to maintain visits to 10 of the 17 farms to obtain BCS at calving, so tests on the influence of this variable on reproductive or productive performance were made only in 7 farms. Only a few inseminations were done under the same conditions with each semen batch, a fact which prevented a more detailed analysis of effects of semen on fertility.

4.1. Survey of reproductive parameters using the radioimmunoassay of progesterone

The progesterone assay on milk samples proved to be a useful diagnostic tool. Three samples, together with the result of rectal palpation (Table II) gave a clear picture of the efficiency of AI, which was around 47% conception to a single insemination. This CR is good compared to other reports (e.g. in USA: 40.9% for 19 herds in N. York State in 1995; 36.9% for 39 herds in 1996; 40.9% for 191 165 records in the North-east) [10].

Reasons for reproductive wastage were non-fertilisation and probable loss of the conceptus when fertilisation did occur. Similar results were obtained in a French study where embryo mortality was 22% in 122 cows from 2 farms and 37% in 197 cows from another farm [11]. The information obtained with two milk samples on the day of AI and 10–12 days later (Table I) was less accurate but could be used to diagnose gross failures in heat detection.

The verification of intermediate assay values for milk progesterone concentration by re-assay is of particular interest. The true prevalence of intermediate values appears to be around 8% of the total inseminations. This would include samples collected early or late with respect to ovulation as well as formation of corpora lutea that produced insufficient progesterone to reach the threshold for a "high" classification. When considering only fixed-time inseminations after an induced oestrus, the proportion of intermediate values becomes higher, reinforcing the idea that intermediate progesterone levels are mainly due to wrongly timed inseminations relative to ovulation. Milk progesterone analysis indicated that almost 6% of AI were performed on pregnant or anoestrous cows or those with ovarian cysts. Reimers and co-workers [12] found 5.1% of inseminations performed when cows were not in heat. This value is a mean between farms, but in some individual farms up to 60% inseminations are performed in the same conditions. In another study O'Connor [13] found 10% mid to high serum progesterone at the time of AI. The progesterone assay could be used as a service to farmers on a routine basis if the accuracy of 97.4% for early diagnosis of non-pregnancy could be sustained.

4.2. Reproductive parameters

Winter calving cows are bred during spring. Probably this was the reason for higher FSCR for cows calved in this season. Cows inseminated in spring are the ones with the highest FSCR and higher OCR than in winter. The effect of season includes many factors such as temperature, humidity, hours of light and pasture growth, that could explain the higher fertility but were not recorded. Cows tend to conceive in spring when they are not forced to become pregnant in other seasons. The deleterious effects of high temperatures on embryo development have been intensively studied in the past [14] and seem to have been confirmed here through the low conception rates obtained in summer (23.5%).

Summer calving cows had the longest interval to first insemination and to conception (Table III). Mean BCS at calving for summer was 2.75 (Table V). In a season of scanty forage, these animals had to face peak production with low body reserves. In spite of the fact that CSI was not related to body condition at calving, the gap between energy intake and requirements for milk production could have been large, leading to a delay in resumption of ovarian activity. It is also possible that a farmer's decision to not inseminate cows during the hot weather, could have contributed to longer CSI.

Spring calving cows also had a long CCI. The ones calving at the beginning of this season could be bred in the same season, but those calving after mid November had to be bred in summer, suffering the effects of the hot weather, or wait until next autumn. They had to pass their first months of lactation during the summer months, and had the lowest BCS at the time of breeding. The lack of energy during this period could not only delay the onset of ovarian activity but also induce lower FSCR compared to winter calving cows that had better BCS at the time of their first insemination (Table V). The effect of body reserves on conception rates is controversial. Previous work by Domecq and co-workers [15] demonstrates that the change in body condition during the first lactation month is the relevant factor influencing CR. They found that cows that lose 1 SD or 0.4 points of body condition in this period are 1.17 times (1/odds ratio) more likely to conceive than the ones that loose less weight. Recently, Diskin [16] found lower CR when energy in the diet was reduced prior to AI. This effect is probably mediated through the progesterone metabolism, but with the present methodology it was not possible to confirm this assertion. In fact, there was no relationship between BCS at calving or at the time of the first AI on conception rates.

The group P+C had higher BCS at calving and lower BCS at the time of AI than the one fed the three types of feed (Table IX). The greater body condition lost between calving and first service might be due to a lower intake capacity in the group P+C (they were fatter at calving and ate less fibre). Both groups had shorter CSI and CCI than the P+H group, probably because of a higher energy status, though this is not clear from the present results because BCS at calving could not be measured in cows consuming P+H. The correlation between BCS at AI and CSI could only be due to an improvement of body stores as time elapsed since calving.

Differences in CR between the types of feeding used here are likely to be due to the differences in the quality or quantity of their protein. Previous work has demonstrated direct or indirect deleterious effects of circulating urea on CR by altering uterine environment or through the influence on milk production and/or energy balance [17]. As it was shown in Argentinean grazing dairy cows, requirements of degradable protein are largely covered by pastures [18]. Additional degradable or undegradable protein from concentrate sources contribute to higher urea levels when there is a lack of energy in the rumen for bacterial protein synthesis, or in the liver, necessary for urea detoxification [19]. The higher milk urea found in cows fed P+C with or without hay compared with those that were fed only P+H, could reflect this particular nutritional situation, which was related to the insemination outcome: higher milk urea in barren cows, specially in winter.

The technician was shown to be one of the most important factors related to insemination outcome, specially those traits related with skill, like formal training and a tendency of intermediate experience to have better performance. Technicians with less than 10 years and more than 20 years of experience tended to have lower OCR (Table VIII). This may be related to the time necessary for the acquisition of abilities for the insemination techniques and points to the need of re-training programmes for inseminators, as has been shown by Senger and co-workers [20].

Incentives might improve results of AI. Only a few technicians were included in each type of payment method and it's influence on technician performance is not clear from the data in this study. Technicians who earn fixed salaries were employed by government institutions (research centres or agrarian schools), and could probably be better trained.

Argentinean dairies seldom are family enterprises. The farm work is generally left in the employee's hands. This could be why the relationship between family income derived from dairying and reproductive performance is not consistent. It could be assumed that increments of income derived from dairying would imply more dedication and better results. On the contrary, during the present study, CRs were lower when income was derived nearly 100% from dairying. The relationship between time dedicated to dairying and CR was inconsistent. Performance is more a matter of efficiency of the work than of the time spent. Similarly, efficiency is likely to be related more to skill and training than to family income.

During prooestrus, uterine tone increases with oestrogen secretion prior to ovulation and is maximum when the cow shows sexual receptivity [6]. In our study, inseminations made approximately 11 h after heat detection, when uterine tone was marked (Fig. 1), resulted in lower CR. This might indicate that some inseminations were performed too late in relation to ovulation. Ovulation occurs approximately 24 hours after heat initiation and heat detection schedules seldom allow to determine when heat begins. After a thorough review of the causes of low CR, Wiltbank [10] concluded that it appears to be better to breed too early than too late in relation with ovulation. In this study CR was not related to time elapsed since heat detection. Our results suggest that frequency of heat detection should be increased to know the moment of heat initiation or that cows should be bred as soon as they are detected in heat at least in farms suspected of low conception rates due to wrong timing of inseminations.

Ninety one percent of 425 inseminations were performed after heat detection based on standing behaviour. The low number of inseminations performed after heats detected based on secondary heat signs does not allow strong conclusions to be made on this factor. A more detailed study of technician performance in relation to heat detection based on secondary signs, including uterine tone and presence and type of mucus discharge, might help to determine the proper time for insemination and to decrease chances of conception failure.

Semen classified as "good quality" was demonstrated to be more fertile than those unexamined or without good information on quality. Twelve from 15 inseminations performed with semen classified as "not good quality" resulted in pregnancy. In spite of this low number of observations it is clear that the available methods of assessment are not accurate enough to predict fertility. The development of objective and accurate indices of sperm fertility would be a great advantage to improve reproductive performance.

5. CONCLUSIONS

Progesterone measurement in milk samples taken on days 0, 11 and 22 of the oestrus cycle was a very useful tool for the differentiation between non-fertilisation and late embryonic mortality, and of improper time of insemination, as causes of non-pregnancy. Accuracy of non-pregnancy diagnosis through milk progesterone in the present study was over 97%. Overall CR was 42.6% and percentage of embryo mortality was 10%. Inseminations performed in the presence of improper progesterone values was 6% for spontaneous oestrus periods and 17% for fixed timed inseminations after synchronized oestrus.

Supplemental protein from concentrates could have produced higher milk urea levels and these were associated with lower CRs. Cows that were fed only with grass and roughage had longer CSI and CCI but better CR. An economic analysis must be done to determine if this type of management is profitable under the present Argentinean conditions. Technician training is considered a priority in order to achieve improvements in conception rates. Marked uterine tone at the time of AI may indicate a delay in timing of insemination and was associated with lower CR. The site of deposition of semen was clearly related to CR. The need for an objective system for semen evaluation was identified.

Areas for future research include: dietary energy and nitrogen balance in the different seasons to obtain higher milk production, better CR and shorter intervals to service and conception; pharmacological control of oestrous cycles to shorten the intervals to first service and conception in cows calving in spring and summer; ways of recognizing the proper timing of insemination including secondary heat signs and uterine tone; and use of improved semen evaluation methods.

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USE OF NUCLEAR TECHNIQUES FOR EVALUATION OF FIRST SERVICE CONCEPTION RATE IN DAIRY HERDS WITH ARTIFICIAL INSEMINATION IN CHILE

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Abstract

USE OF NUCLEAR TECHNIQUES FOR EVALUATION OF FIRST SERVICE CONCEPTION RATE IN DAIRY HERDS WITH ARTIFICIAL INSEMINATION IN CHILE.

The objective of this study was to identify causes of inefficiency in Artificial Insemination (AI) services in 12 dairy farms located in southern Chile. Milk progesterone concentration was determined on the day of breeding and then 10–12 and 21–22 days after AI. Data for semen and cow inseminated, including physical signs of oestrus, were recorded in a computer database (AIDA). Information from 713 cows with first services was analysed. The mean interval from calving to first service was 88.7 days and the mean interval from calving to conception was 107.9 days. The conception rate at first service was 61.9%. Incidence of incorrect AI, most likely due to erroneous heat detection, was 8.9%. Herd related problems affected efficiency of AI in 15.2%. The results show that important factors affecting reproductive performance include nutritional management, oestrus detection and AI technique.

1. INTRODUCTION

Milk production in Chile is concentrated mainly in the southern region (Regions IX and X) and has shown a rapid growth during the past decade, rising from 1300 million L in 1990 to 2030 million L in 1998 [1]. The intensification of dairy systems in Chile is responsible for this improvement, and has required the introduction of new programmes for genetic improvement, artificial insemination (AI), feeding and hygiene to achieve high performance levels.

Reproductive efficiency in dairy herds has a marked effect on profitability. Several factors influence reproductive performance, the important ones being accurate detection of oestrus, correctly timed inseminations, fertilizations, early embryonic development and maintenance of pregnancy [2]. Many recent studies have shown that under modern management and housing systems, inaccurate detection of oestrus and early embryonic death are major problems. In the former case, breeding other than around the time of oestrus may account for between 5–12% for all services and may be as high as 20–30% under certain management systems [3].

Under these circumstances, not only are the cost of maintaining the cows and purchasing and holding semen wasted, but other reproductive problems including early embryonic death, may result from incorrectly timed inseminations [3]. Under farm conditions, fertilisation rates as high as 75% are probably achieved, but on day 40 after AI, only 53% of cows are likely to be pregnant [4].

A reliable management tool to monitor reproductive function in dairy cows would greatly improve their reproductive efficiency. The concentration of progesterone in milk is closely related to the oestrus cycle of dairy cows. The accurate determination of the progesterone levels can be used to confirm oestrus and diagnose non-pregnancy as well as to monitor postpartum ovarian activity, early embryonic death and ovarian disorders [2, 5, 6, 7, 8, 9].

We conducted a field study to obtain information on the reproductive management during three years in twelve commercial dairy herds in southern Chile with the aid of nuclear techniques for measuring progesterone levels in milk. The objectives were to establish current status of fertility in 12 dairy herds subject to AI and to identify relationships between cow characteristics and the overall conception rate at first service.

2. MATERIALS AND METHODS

2.1. Location

The study was conducted during May 1995 to December 1997 in twelve dairy farms located in Region IX of Chile (38°Lat.S). Table I contains the characteristics of different farms involved in this study.

2.2. Milk samples and data collection

Farmers were provided with plastic sampling vials containing milk preservative (sodium azide tablet) and were asked to collect foremilk samples on the day of breeding, and then 10–12 and 21–22 days later. Samples were refrigerated until collected for analysis within 1–2 weeks. A questionnaire on general information of farms and reproductive management was distributed. Farmers and the technicians doing the inseminations were asked to record the identification of the semen and the cow at insemination, including physical signs of oestrus.

2.3. Radioimmunoassay of progesterone in milk

Milk progesterone concentration was determined using a solid phase radioimmunoassay (DPC, Los Angeles, USA) [7]. The tubes were counted for radioactivity on a gamma counter. The coefficients of variation within and between assays were 5.6% and 8.4%, respectively.

2.4. Data analysis

Data from farms were entered into the Artificial Insemination Database Application (AIDA), a computer software package designed to store data and provide reports related to AI [10].

First service conception rate (FSCR) resulting from 713 first services was analysed considering the following as variation factors: farm, breed, parity, season of calving, season of AI, oestrus manifestation, oestrus detection to AI interval, calving-to first service interval (CFSI), calving to conception interval (CCI), and some aspects of the AI technique. The data was then analysed using SAS and SYSTAT.

3. RESULTS

3.1. Farm description

Table I shows the characteristic of each farm utilised in this study. The following farm variables were used in a cluster analysis to characterise the production systems: total land (ha), grazing paddocks (ha), crops (ha), total number of cattle, number of milking cows, stocking rate, average milk production, farm income and % of concentrate in the feed. The characterisation of production systems determines the interaction of endogenous factors with productive levels. Due to this interaction it is feasible to expect defined patterns in production levels.

Four types of farms were created by cluster analysis. Cluster 1 with 7 farms: Chivilcan, Los Aromos, Maipo, Palihue, Ranchillo, Santa Rosa and Santa Elena. These farms are smallest in size, both in land and number of cows. Almost 50% of the land is for crop and 50% for pasture, with a relatively high stocking rate on pasture (1.46 head/ha). Cluster 2 was formed with three farms: Huilquilco, Pichiquepe and Santa Margarita. This cluster belongs to medium size farms, also intensive

(1.47 head/ha), but with high numbers of cows in milk production. Clusters 3 and 4 were formed by one farm each: Calatayud and San Bernabe. Calatayud was the biggest farm, both in terms of cattle and land and with the highest milk yield (8000 kg). Nevertheless the stocking rate is low (0.56 head/ha), dairy is the main activity; less than 6% of the land was used for crops. On the other hand San Bernabé is the farm with the lowest milk yield; 50% of the land was used for crops and the stocking rate is very low (0.33 head/ha).

TABLE I. CHARACTERISTICS OF FARMS USED IN THE STUDY

Farm	Total land (ha)	Pasture (ha)	Total cattle	Milking cows	Avg. milk yield (L/cow/day)	Concentrate feed (kg/cow/d)
Calatayud	640	450	730	480	25	6
Chivilcan	246	123	221	74	186	3
Huilquilco	454	368	470	248	24	4
Maipo	138	40	120	40	244	6
Los Aromos	110	20	95	30	215	4
Palihue	150	75	47	20	145	2
Pichiquepe	260	234	200	125	20	4
Ranchillo	67	40	61	25	185	4
San Bernabé	600	300	140	70	11.6	0
Santa Elena	148	60	225	84	194	4
Santa Margarita	250	194	251	190	21	4
Santa Rosa	74	60	127	70	226	6

Table II shows FSCR on the different types of farms for 713 observations. There were statistical differences between all farms, with the highest CR in farms of type 3.

TABLE II. NUMBERS OF COWS INSEMINATED (N) AND FIRST SERVICE CONCEPTION RATE (FSCR) IN THE FOUR TYPES OF DAIRY FARMS ACCORDING TO CLUSTER ANALYSIS

Farms	Number of cows inseminated	FSCR (%)
Farm type 1	186	55.3[a]
Farm type 2	356	51.4[b]
Farm type 3	139	75.7[c]
Farm type 4	32	68.8[d]
Total	713	61.9

[a, b, c, d] Different letters in the same column indicate significant differences (P <0.05).

3.2. Seasonality

Cows that calved in the spring and winter had a higher FSCR than cows calving at other times (Table III).

TABLE III. FIRST SERVICE CONCEPTION RATE (FSCR) IN COWS CALVING DURING DIFFERENT SEASONS

Calving Season	Total first AI	Number conceived	FSCR (%)
Spring	75	51	68.0[a]
Summer	163	89	54.6[b]
Autumn	315	190	60.3[b]
Winter	160	111	69.4[a]

[a, b] Different letters in the same column indicate significant differences (P <0.05)

Cows that were inseminated during spring and summer had higher FSCR than others (Table IV).

TABLE IV. FIRST SERVICE CONCEPTION RATE (FSCR) IN COWS INSEMINATED DURING DIFFERENT SEASONS

AI Season	Total first AI	Number conceived	FSCR (%)
Spring	159	115	72.3[a]
Summer	59	43	72.9[a]
Autumn	296	161	54.4[b]
Winter	199	122	61.3[b]

[a, b] Different letters in the same column indicate significant differences (P <0.05)

3.3. Parity

There was no statistical difference in the FSCR among cows of different parity numbers (P >0.05). There was a difference (P <0.05) between types of parturition, with higher FSCR in cows having a normal calving (69.2%) compared to that in cows with obstetric problems at calving (45%).

3.4. Heat expression

Cows inseminated at standing heat had a higher FSCR (P <0.05) when compared to cows showing only mounting behaviour (56.3% vs. 67.7%). FSCR was highest in cows with a markedly coloured (hyperaemic) vulva (71.4%; 210/294), intermediate in those with a slightly coloured vulva (65.9%; 83/126) and lowest in those with no colouration (38.5%; 10/26) (P <0.05). No statistical difference was found when comparing the FSCR of cows with normal mucus (69.3%) and other types of discharge (64.1%) (P >0.05).

3.5. Time of AI

Fig. 1 shows the FSCR in cows inseminated at different times after the detection of oestrus. Cows inseminated 18 hours after detection of oestrus had the highest FSCR (81.9%) and this was significantly different (P <0.05) from the FSCR in other categories.

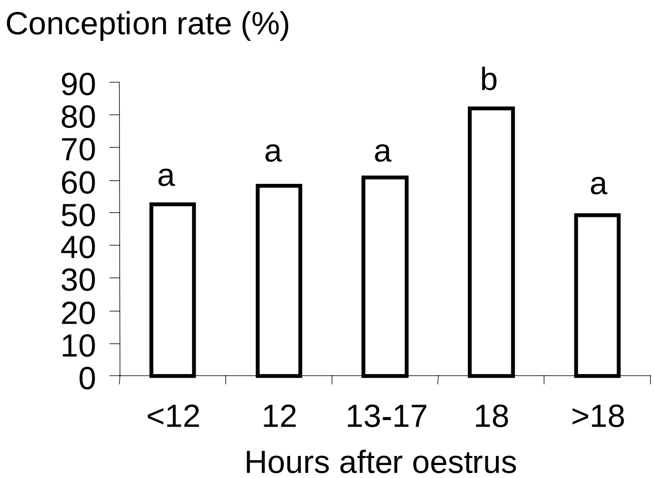

FIG 1. Effect of interval between oestrus to AI on first service conception rate in 441 milking cows.

Cows inseminated during the afternoon had a higher FSCR (72.9%; 231/383) compared to cows inseminated in the morning (60.3%; 151/207) (P <0.05). No statistical differences (P >0.05) were seen between FSCR of cows inseminated with difficulty (69.3%; 277/400) or with ease (62.3%; 38/61), and between cows inseminated in the uterus (69.2%; 299/432) or in the cervix and vagina (55.2%; 16/29).

3.6. Semen characteristics

There were no differences in FSCR achieved with semen from Friesian bulls (70.4%; 57/81) or with semen from Holstein-Friesian bulls (60.8%; 384/632) (P >0.05). No statistical differences were found between FSCR from semen of local AI centers (60.2%) or imported semen (62.1%), and between semen straws of volumes 0.25 or 0.5 mL.

3.7. Progesterone RIA

Using progesterone values based on three milk samples taken on days 0, 10–12 and 21–22 after AI in conjunction with clinical examination (rectal palpation), it was possible to interpret the reproductive status of the cows as shown in Table V. Thus, 75.8% of the cows were pregnant after AI, 9.4% were non-pregnant and 4.7% had late embryonic mortality.

TABLE V. PROGESTERONE DATA INTERPRETATION: DIAGNOSIS BASED ON THREE SAMPLES

Days after AI			Pregnancy diagnosis	Frequency		Interpretation
0	10–12	21–22		n	%	
Low	High	High	+	194	75.8	Correct AI resulting in pregnancy
Low	High	Low	-	24	9.4	AI in follicular phase, conception failure and subsequent oestrus not detected
Low	High	High	-	12	4.7	Embryonic mortality
High	High	High	+	8	3.1	AI on pregnant cows
Low	Low	Low	-	3	1.2	AI in anoestrus cows

Total values n = 332. Intermediate values (>1 to <3nmol/L) n = 76

In some cases, however, only the first sample or the first and second samples were available. The interpretation of data in such cases is shown in Tables VI and VII. Based on two samples (day 0 and 10–12 after AI) 3.9% of the cows were inseminated in anoestrus and 5.9% were inseminated while pregnant or having luteal cysts. Based on one sample (day of AI), it was possible to determine that 5% of the cows were inseminated during the luteal phase.

TABLE VI. PROGESTERONE DATA INTERPRETATION: DIAGNOSIS BASED ON TWO SAMPLES

Days after AI		Frequency		Interpretation
0	10–12	N	%	
Low	High	394	90.2	AI in follicular phase
Low	Low	17	3.9	AI in anoestrus cows
High	High	26	5.9	AI in pregnant cows
High	Low	0	0	AI in luteal phase

Total values n = 564. Intermediate values (>1 to <3nmol/L) n = 127

TABLE VII. PROGESTERONE DATA INTERPRETATION: DIGNOSIS BASED ON ONE SAMPLE

Day 0	Frequency		Interpretation
	N	%	
Low	612	95.0	AI in cows without active corpus luteum
High	32	5.0	AI in cows with active corpus luteum

Total values n = 739. Intermediate values (>1 to <3nmol/L) n = 95

4. DISCUSSION

The analysis of the information obtained from 713 first services shows that interval from calving to first service was 88.7 ± 56.7 days and the interval from calving to conception 108 ± 73.3 days. Differences between the farm management systems explain some of the differences seen in these parameters. A previous study [8] described 68.8 days for calving to first oestrus and 81.6 days for calving to conception in a dairy herd of the same region. This indicates that both intervals have increased by about one oestrous cycle length during a period of ten years.

FSCR was 61.9%. In 1990 Correa and co-workers [8] found a similar rate (60%). This indicates that the factors affecting these parameters did not change in the past years. Thus the longer anoestrus post partum in these herds could be due to a negative energy balance during the first months after calving. The intensification of milk production has put pressure on the reproductive efficiency because the demands of energy are very high during the first 3 months of lactation and at the same time cows need to become pregnant [11, 12, 13].

In this study the season during the time of calving and AI affected the FSCR, with higher rates were obtained in cows calving during winter and spring. Farmers improve the feed during these periods because they obtain competitive prices for the milk, and this results in better body reserves in the cows during winter and spring [14, 15]. This effect is reflected during the AI period (about 3 months after calving) because in spring and summer the FSCR was also better compared with other seasons [12].

No differences in FSCR were found in cows with different lactation numbers; this is not common because others studies show cows at first lactation with a low FSCR [12, 15]. FSCR was low in cows with problems at parturition; the possibility of infections during calving in these cows with dystocia is high and affect the survival of semen in the reproductive tract [14].

Standing to be mounted by other cows (standing heat) is considered to be the most reliable sign for diagnosis of oestrus in the cow [16]. The cows that showed only mounting of other cows had a low FSCR; such cows may not be in oestrus, or still in the pro-oestrus period. Hyperaemia of the vulva was also a heat sign associated with high FSCR. This indicates that the intensity of oestrus is an important parameter for success in AI [15, 16]. Higher FSCR was obtained when the AI was done 18 hr after the onset of oestrus; thus farmers should be advised to get their cows inseminated as close as possible to this timing. The finding that FSCR was higher when AI was performed in the afternoon rather than in the morning is difficult to explain, but factors such as higher pressure of work during the morning could have had an influence.

The progesterone analysis was valuable in determining the proportion of inseminations done at the incorrect time, and also for detecting late embryonic death in cows that had conceived. Overall, the study showed that failure to detect oestrus in normal-cycling cows is an important factor affecting reproductive performance in dairy herds. Therefore, the urgent need is to improve the knowledge of farmers on oestrus behaviour and its relation to the optimal time for performing AI.

5. CONCLUSION

The principal factors affecting the FSCR in the farms studied were heat expression, body condition at calving, season of calving and AI, and timing of AI in relation to onset of oestrus. Factors such as semen volume (0.25 or 0.5 mL), source of semen (local or imported) and breed of bull (Friesian or Holstein) did not affect the FSCR. Measurement of progesterone by RIA at 0, 10–12 and

22 days after AI permitted an accurate assessment of the reproductive status of cows and outcome of AI. Around 9% of inseminations were done at the wrong time and of those done at the correct time about 15% did not result in pregnancies due to problems related to herd management.

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IMPROVING PRODUCTIVITY THROUGH THE USE OF ARTIFICIAL INSEMINATION IN DUAL PURPOSE FARMS IN COSTA RICA

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Abstract

IMPROVING PRODUCTIVITY THROUGH THE USE OF ARTIFICIAL INSEMINATION IN DUAL PURPOSE FARMS IN COSTA RICA.

The objective of the present study was to identify the major causes of inefficiency in the AI services provided to dual-purpose farms in the region of Tilarán, Guanacaste. The study included four representative farms from the region, where AI was done on a routine basis in which 80 to 100 first services were done annually. The overall conception rate (CR) was 42.7% (271/635) and was significantly influenced by three variables: lactation, oestrus signs and technician. Cows that were inseminated during their lactation number 5 had 2.17 times greater chance of getting pregnant ($P < 0.001$) than cows inseminated during any other lactation. Cows inseminated after detecting oestrus because they were mounting others had 1.2 less chance to get pregnant than those detected by standing heat, but those cows detected by other signs such as restlessness or bellowing had 1.8 more opportunity to get pregnant than those detected by standing heat. The interval from calving to first service was 114.1 days and three variables had significant ($P < 0.05$) effects on this interval: farm, calving season and lactation. Cows calving during the rainy season had a shorter interval than those calving in the dry season. There was a significant difference between the first lactation and the others. Only lactation had significant effect on first service CR. It was concluded that farm type, lactation, season, and heat signs were the most important factors having an influence on the efficiency of AI. Variables involved are closely related to management and should be targeted in future work aimed at improving reproductive efficiency.

1. INTRODUCTION

Milk and beef production are important components of the Costa Rican economy, but cattle are also thought to be the major cause of forest and land degradation. Given the current human population growth rate (2.6% per annum) and using estimates of the actual crop yield per hectare, all of the one million ha suitable for production of annual crops in Costa Rica would be needed to produce the calories required to support the human population by about 2030 [1]. Since only 325 000 ha are currently in annual cultivation, this would mean that nearly 700 000 ha of land now used for pasture and woodlands would need to be brought under cultivation [2]. One potential solution is to increase the efficiency of cattle production systems, with the objective of reducing the number of animals and area of land utilized by cattle. Productivity and efficiency are central factors in sustainability. As productivity is enhanced, fewer resources are required for the same output. Increased economic efficiency should lead to the conservation of resources [3].

Artificial Insemination (AI) is one of the techniques that can lead to greater efficiency of production. It is widespread throughout the country especially in dairy cattle areas. Increasingly it is being used in dual purpose and beef enterprises. The efficiency of AI in dual purpose farms is considered to be low, many farms are small and the farmers lack education [4]. The objective of the present study was to identify the major causes of inefficiency in the AI services provided to dual purpose farms in the region of Tilarán, Guanacaste.

2. MATERIALS AND METHODS

2.1. Region

The Tilarán region was selected because there was already a Herd Health Management and Production Programme (HHPM) established by the School of Veterinary Science. This and the presence of AI services provided the necessary infrastructure for the survey. Tilarán is part of a tropical moist forest area with an average rainfall of 2000 to 4000 mm and ambient temperature between 24–30°C. Dual purpose farms in the region are an average of 75 ha (range 5–252 ha). The mean farming experience of the managers was 16 years. On average one adult unit of labour is used for each 44 cows (range 7–164). Family labour is primarily used for management activities such as milking, calf rearing and feeding practices. Nutrition is pasture based on Star grass (*Cynodon plectostachyus*) combined with native grass and Jaragua (*Hyparrhenia rufa*). The average number of paddocks is 17 (range 2–83) with a stocking rate of 1.82 animals per ha (range 0.61–2.34). Supplementation with molasses and mineral salt often occurs during the dry period. (December to May). The average calving rate is 46% (range 38–57). Milk production ranges from 1500–2000 kg per lactation of 270 days [5].

2.2. Farms

The study included four representative farms from the region, where AI was done on a routine basis and where 80–100 first services were done annually. These farms were already using HHPM assistance. They were visited monthly by a veterinarian, for activities associated with reproduction. Reproductive events were being recorded and stored using a computer program. This program includes all the farm information and individual information from each cow such as: animal identification, breed, date of birth, date of parturition, type of parturition, body condition, body weight, name of technician, date of service, sire and semen batch, medical treatments and other comments.

All cows were inseminated by private technicians using semen bought from commercial companies. Pregnancy diagnosis was done on the routine visit, 35–45 days after AI.

Information about oestrus and insemination was recorded on a special protocol which included date and time when oestrus was first observed, clinical signs, time of service, site of deposition of semen, reasons for no service and treatments.

2.3. Milk sampling and assay of progesterone

Milk samples were taken from all inseminated cows on day 0 (day of insemination) and days 10 and 22–24 after AI. Samples were collected by the farmers or their employers and refrigerated at the farm. Twice a week samples were picked up by project staff members, centrifuged to remove fat and the fat-free milk frozen until processing for progesterone determination. Progesterone was measured using RIA kits based on a non-extraction solid-phase assay using ^{125}I-labelled progesterone supplied by the International Atomic Energy Agency (IAEA, Vienna). Inter and intra-assay coefficients of variation were 13.5 ± 2.0 and 3.5 ± 2.9.

2.4. Data analysis

All information concerning insemination, cow inseminated, and farm as well as the progesterone value was recorded in the Artificial Insemination Database Application (AIDA, Joint FAO/IAEA Division, Vienna). The application was developed using Microsoft Access 2.0 for Windows™ [6] to store data and provides reports related to the AI service. Information collected was first analyzed to evaluate the quality of the AI service and to identify constraints that may affect fertility. Also, progesterone concentrations in milk samples were correlated with fertility to facilitate data interpretation. AIDA was used to obtain data output for Microsoft Excel files which were then used for a multiple regression analysis of factors likely to be affecting efficiency [7].

Three variables were used for breed: *Bos indicus*, *B. taurus* and crosses of *B. indicus* and *B. taurus*. Lactations were numbered 1 (cows with one or less lactations) to 7 (cows with 7 or more lactations). Parturition type was described as normal or abnormal (requiring assistance). Behavioral signs of oestrus were classified as "standing heat", "mounting others" and "other signs" (restlessness, bellowing and the presence of clear vaginal mucus). The type of mucus discharge was described as clear, turbid or none. Site of deposition of semen was classified as "cervix" or "other sites". The ease of passage of pipette through cervix was described as "easy" or "difficult". Calving and insemination season was classified as "rainy" or "dry".

Inseminations were classified as "correct" or "incorrect" based on progesterone values in the 3 milk samples. Incorrect was when progesterone concentrations were high (<1.5 nmol/L) on day 0, or low (<1 nmol/L) on day 0 and day 10, or low on all three sampling days. For the characterization of the farms a Proc Cluster Analysis was used. This is a multivariable technique that splits a set of farms into a selected number of groups by maximizing between cluster variation and minimizing within cluster variation [7].

To establish potential relationships between farm and cow characteristics with the overall conception rate (CR), the first service CR, the interval from calving to first service (CFSI) and the interval from calving to conception (CCI), a first descriptive analysis was used. Then with the potential risk factors and the CR a univariate analysis was performed. With those variables that showed biological or statistical significance in the previous analysis a multivariate analysis using logistic regression and survival analysis was performed.

Finally a Cox Model, a proportional hazard model, was used to describe the risk of each variable. Age and lactation number were highly correlated (0.80, P <0.001) so in the analysis of results only lactation was used as a variable. Common to all four farms were the use of commercial frozen semen, the system of semen storage in a liquid nitrogen tank, the use of visual heat detection without other aids, primary school level of education of technicians with no formal training on AI, and a fixed salary method of paying technicians who also had other farm duties to perform. Hence these variables were not used in the model.

3. RESULTS

3.1. Cluster analysis

The cluster analysis created three types of farms (Table I). Cluster one contained 2 farms: Ayotes and Tierras Morenas. Both farms were located at Tilarán, were dual purpose, with once a day milking session and suckling by calves for milk let down. Family income from cattle farming ranged from 26–50% and the time they spent on the farm was 51 to 75 %. Clusters two and three were formed with one farm each, Fuentes y Alfaro and La Pacífica respectively. Their characteristics are shown in Table I.

TABLE I. CHARACTERIZATION OF THE FOUR FARMS BY CLUSTER ANALYSIS

	Cluster 1	Cluster 2	Cluster 3
Total cattle	178	211	723
Breedable cattle	55	90	383
Bulls	1	1	10
Weaning age (m)	7	0	8
Location	Tilarán	Tilarán	Cañas
Purpose	Beef-dairy	Dairy	Beef-dairy
Suckling	Once (Let Down)	None	Once
Milkings/day	1	2	1
Family time on farm	51–75 %	51– 75 %	0–25 %
Family income from farm	26–50 %	51– 75 %	0–25 %

3.2. Reproductive performance

The reproductive performance of the whole population surveyed is presented in Table II. The average age of these cows was 10 years, with a standard deviation 12.3, and an average of 3.6 lactations with a standard deviation of 1.8.

TABLE II. REPRODUCTIVE INDICES FOR CATTLE ARTIFICIALLY INSEMINATED ON FOUR FARMS IN COSTA RICA

Reproductive Index	N	Mean ± SD
Overall conception rate (%)	635	42.7
First service conception rate (%)	486	41.9
Services per conception	658	3.5
Interval from calving to first service (days)	500	114.1 ± 78.8
Interval from calving to conception (days)	186	138.8 ± 93.8

3.2.1. Overall conception rate

There were 635 usable observations for the analysis and the overall CR was 42.7%. Three variables showed a significant effect on this parameter (Table III). There was no significant effect of cluster, farm, breed, calving or insemination season, site of semen deposition, passage of pipette, type of mucus or parturition type.

TABLE III. GENERAL LINEAR MODEL OF FACTORS AFFECTING THE OVERALL CONCEPTION RATE

	DF	Mean Square	F value	P value
Lactation	6	18 880.3	2.30	0.03
Oestrous signs	2	29 005.9	3.53	0.03
Technician	3	19 804.7	2.41	0.06

Cows that were inseminated during their lactation number 5, had 2.2 times greater chance of getting pregnant ($P < 0.001$) than the cows inseminated during lactation 1. Signs used to detect oestrus had a significant effect ($P < 0.05$), cows inseminated after detecting oestrus because they were mounting others had 1.2 less chance to get pregnant than those detected by standing heat, but those cows detected by other sign such as restless or bellowing had 1.8 more opportunity to get pregnant than those detected by standing heat. The technician variable was included in explaining the model ($P = 0.068$), but there was no difference between technicians.

3.2.2. Interval from calving to first service

There were 500 usable observations for first services and the CFSI was 114.1 days (Table II). The average age for these cows was 6.7. With the general linear model (GLM) with a backward procedure looking for a simple explicative model, three variables had significant effect on CFSI while the others did not (Table IV): farm cluster, calving season and lactation. Cluster 3 had a shorter interval (106.1 days) than clusters 2 and 1 (143.7 and 132.1 days respectively, $P < 0.05$). Cows calving during the rainy season had a shorter CFSI (118.8 days) than those calving in the dry season, (135.8 days). Figures 1 and 2 with a Kaplan-Meier survival functions illustrate the effects of farm and season respectively. Cows in lactation one had a longer CCI ($P < 0.05$) than those in other lactations (Table V).

TABLE IV. GENERAL LINEAR MODEL OF FACTORS AFFECTING INTERVAL FROM CALVING TO FIRST SERVICE

	MS	F-value	P-value
Calving season	28 031.6	4.47	0.03
Lactation	24 303	3.87	0.04
Farm cluster	19 552.2	3.11	0.04

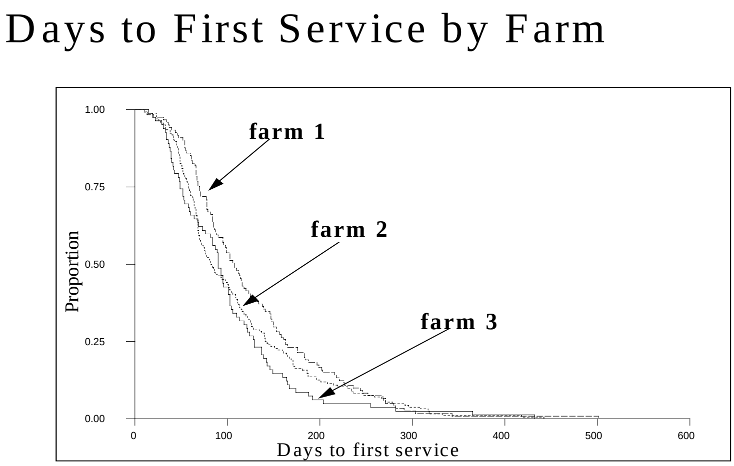

FIG.1. Effects of the farm cluster on the interval from calving to first service.

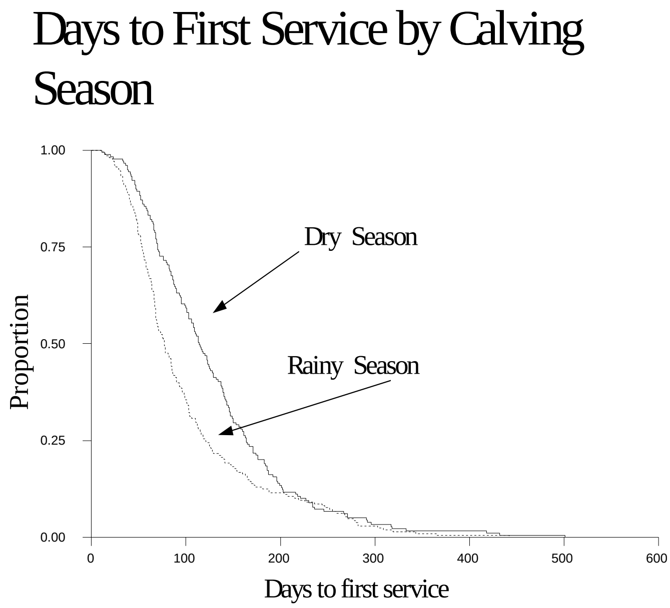

FIG. 2. Effects of calving season on the interval from calving to first service.

TABLE V. LEAST SQUARE MEANS FOR LACTATION

Lactation	Mean	P value
1	163.7	0.03
2	124.5	0.8
3	129.3	0.8
4	115.5	0.4
5	100.8	0.1
6	126.2	0.9
7	127.2	0.9

3.2.3. First service conception rate

First Service CR for 486 cows having an average of 10 ± 13.9 years and 3.6 ± 1.8 lactations. was 41.9% (Table II). With the general linear model only lactation ($P < 0.05$) had a significant effect. The results of a proportional hazard model, to try to consider the relationship of the variables and their influence on the time of conception at first service are shown in Table VI. The other variables, technician, breed, calving season, time to insemination had no significance effect.

TABLE VI. CRUDE ODDS RATIOS (OR) AND SIGNIFICANCE OF FACTORS AFFECTING FIRST SERVICE CONCEPTION RATE ACCORDING TO THE PROPORTIONAL HAZARD MODEL ANALYSIS

Variable	Level	OR	CI	P-value
Farm cluster	2	0.79	0.56–1.1	0.19
	3	0.55	0.32–0.94	0.03
Lactation	2	1.08	0.66–1.77	0.74
	3	1.03	0.61–1.73	0.90
	4	1.40	0.84– 2-33	0.19
	5	2.22	1.31–3.77	0.03
	6	1.08	0.57–2.02	0.80
	7	1.08	0.61–1.90	0.77
AI season	2	0.70	0.51–0.95	0.02
Pipette passage	2	0.43	0.19–1.00	0.05
Oestrous signs	2	0.83	0.59–1.17	0.29
	3	2.38	1.26–4.50	0.007

Cows in farm cluster 3 had 1.8 less probability of getting pregnant at first service than those in cluster 1 ($P < 0.05$). A difference was seen only during lactation 5, when cows are 2.2 times more likely to become pregnant than during lactation 1 ($P < 0.05$). Cows inseminated for the first time during the dry season had 1.4 more chance of getting pregnant with first service than those inseminated during the rainy season ($P < 0.05$). Cows with mounting behavior at the moment of first insemination had a tendency to decrease the probability of getting pregnant 1.2 times. On the other hand, cows inseminated when the oestrus sign was other than standing heat or mounting others, such as restless or bellowing had 2.4 more chances of getting pregnant ($P < 0.05$). Finally, cows reported as difficult to inseminate had 2.3 less chance of becoming pregnant than those reported as easy inseminations.

3.3. Progesterone radioimmunoassay results

There were 278 inseminations for which three milk samples were available for assay. The results showed that 54.3% of AI were performed when progesterone was low and in cycling cows, and therefore were performed correctly. In 21.2% of cases the cows had either high progesterone (luteal pahse, pregnant or abnormal), or were anoestrous. In 24.4% of cases at least one sample was in the intermediate range and could not be interpreted (Table VII).

There were 418 inseminations for which two samples were available for assay and these revealed that 60% of AI were performed correctly while 21.5% were done when the cows had either high progesterone or were anoestrous. The incidence of values in the intermediate range was 18%. There were 563 inseminations with only one sample and these showed that 82.7% were performed correctly, 9.4% were incorrect and 7.8% had intermediate values.

Variables were tested with a univariated procedure to see which factors were affecting the accuracy in inseminating cows correctly. No variables seem to be affecting accuracy ($P > 0.05$). Cows that were inseminated correctly based on progesterone levels had 1.83 more chance of getting pregnant than those inseminated incorrectly.

TABLE VII. RESULTS OF RADIOIMMUNOASSAY OF PROGESTERONE IN THREE SAMPLES OF MILK COLLECTED FROM ARTIFICIALLY INSEMINATED COWS

Day 0	Day 10–12	Day 22–24	Pregnancy Diagnosis	Frequency (n)	Percentage (%)	Interpretation
Low	High	High	Positive	91	32.7	Pregnant
Low	High	Low	Negative	30	10.8	Non-pregnant
Low	High	High	Negative	30	10.8	Late embryo death
High	High	High	Positive	15	5.4	AI in Pregnant cows
Low	Low	Low	Negative	44	15.8	AI in anoestrus
Int.	Int.	Int.	Pos/Neg	68	24.5	No interpretation
			Total Occurrence		278	

Low = <1 nmol/L; High = >3 nmol/L; Int. = 1– 3 nmol/L

4. DISCUSSION

4.1. Cluster analysis

The cluster analysis showed that there were three types of farms represented in the four farms chosen. Some of the important differences are considered to be numbers of cattle, suckling management, milking times, weaning age of calf and proportion of family income derived from the farm. This type of analysis and the result obtained here could be important in further characterization of larger sets of farm enterprises in Costa Rica and in making recommendations for improving productivity.

4.2. Reproductive performance

The overall CR found in this study is considered low, as has been previously described in other tropical regions [4]. The intervals from calving to first service and from calving to conception are long and also represent a source of reproductive inefficiency. Reproductive indices in *B. indicus* and their crosses are generally low, particularly in animals raised traditionally [4].

First lactation had a negative effect on CR. There is earlier evidence that first lactations have a negative effect on fertility in the tropical and subtropical zones [8, 9]. It was reported that there must be a genetic component that is influencing the effect of lactation, nevertheless practical experience had proved that with feed supplementation the CR in these animals increases [4]. In the present study no breed effect on overall CR was found.

The results concerning heat signs are of interest in that they differ from those of other workers [10]. It may be that under the management conditions on dual purpose farms in Costa Rica cows are not seen frequently actually standing to be mounted and therefore the restlessness and bellowing recorded here are just as indicative of true oestrus as an observation of standing to be mounted. On the other hand it can be that in *B. indicus* and their crosses other signs rather than standing to be mounted are also important, as previously reported by other workers [11].

Type of farm had a significant influence on interval to first service (Table IV). Management has been found to be important for reproductive efficiency under a wide variety of conditions [12]. During first lactation, which is a time of great stress, there is a significant difference compared to others lactations where stress is handled better by the cow (Table V). Cows calving during the rainy season had shorter interval to first service (Figure 2). Similar findings have been found, were cows that calved during the favorable seasons had better intervals [4, 13, 14]. This is something that can be expected because during the rainy season pasture and nutrition are better.

First service CR was significantly influenced by farm, lactation number, oestrous signs and season. Thus factors related to both management and the cow proved to be important in determining reproductive performance.

4.3. Progesterone radioimmunoassay

The progesterone assay of strategically collected milk samples proved a useful addition to the survey data collection and analysis in defining inefficiencies in the AI services. Despite apparently good oestrus detection too many inseminations (21%) are being conducted at a time when the cow could not possibly become pregnant. This represents a serious waste of resources and steps to improve management need to be taken. Similar work is needed to define why non-fertilization, embryonic death or post AI anoestrus are causing such great losses under many farming systems. Late embryo mortality could be due to infectious diseases and further studies to establish the incidence and prevalence of these diseases would help to increase the reproductive efficiency of these farms.

5. CONCLUSIONS

Lactation, season of calving and insemination, passage of pipette when inseminating, farm and oestrus signs seem to be the most important factors affecting the low CR found in this study. Lactation was the variable that seemed to explain the model best, regardless of the parameter evaluated. First lactation cows were a major problem group responsible for lowering the reproductive indices. Further research must be done to find the causes and remedy them. The signs used to detect oestrus in cows has to be more carefully studied to see if other signs such as restlessness and bellowing can be used effectively under the conditions present on these farms. Season is another variable that has to be considered when trying to improve pregnancy rate and interval from calving to pregnancy. Cows calving during the rainy season, but inseminated during the dry season, have better chances to get pregnant and also to get pregnant earlier. Many of these variables are related to management and this aspect should therefore be the target in future work aimed at improving reproductive efficiency.

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FACTORS THAT AFFECT THE QUALITY AND EFFICIENCY OF ARTIFICIAL INSEMINATION IN OESTRUS SYNCHRONIZATION PROGRAMMES IN DAIRY CATTLE

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Abstract

FACTORS THAT AFFECT THE QUALITY AND EFFICIENCY OF ARTIFICIAL INSEMINATION IN OESTRUS SYNCHRONIZATION PROGRAMMES IN DAIRY CATTLE.

Experiments were performed during a three-year period in order to identify the factors affecting quality and efficiency of artificial insemination (AI) when it is used in cattle subjected to oestrus induction and synchronization (OIS) programmes. The study took place in the Western part of the country and 3 herds with a total of 6357 animals were used. The first study was a survey to evaluate the factors affecting the efficiency of AI in OIS. The second study examined the effects of body condition score (BCS) and three different regimes of OIS on the results of AI. Progesterone levels were measured in blood and milk using radioimmunoassay to determine the incidence of acyclic animals in the herds. Data were recorded and analysed using the artificial insemination database application (AIDA) and further statistical analyses were done using Systat. The results found in the survey showed a low conception rate (18%) and a great variability in the period from calving to conception (154 ± 98.11 days). The latter parameter was significantly different between herds ($P < 0.001$). A high percentage of animals selected for OIS programmes (37%) showed a poor BCS (<2.5 on a scale of 1–5). Motility of semen used for AI was low (<30%) in 47% of the samples evaluated. The results from progesterone measurements demonstrated that the reasons for poor fertility were low accuracy in oestrus detection, poor response to treatment for OIS, non-fertilization, embryo mortality and functional disturbances present after treatment. Under our field conditions during the dry season, acyclicity (anoestrus) is a frequent problem in primiparous cows and in those with poor BCS. The BCS in heifers and the quality of different treatments had a significant effect on the conception rate. These results confirm that the use of progesterone measurement together with data management using AIDA is a very appropriate way to evaluate and improve the quality and efficiency of AI in OIS programmes.

1. INTRODUCTION

Artificial insemination (AI) technology is important for achieving genetic progress in cattle. In 1959 a national programme was initiated to increase milk and beef production through genetic improvement. By using this breeding method the aim was to improve the conception rate (CR) at first service to between 40 to 60% [1]. However, during the past two decades, the CR in AI programmes has been below 45%. Recently, some reports have suggested that the main cause of low CR is the lack of accuracy in oestrus detection [2, 3, 4, 5]. In our country, 30% of cattle bred by AI are subject to oestrus induction and synchronization (OIS) programmes. However, the CR is below 50% [6]. The aims of this project were to identify the causes affecting the efficiency of AI in controlled breeding programmes using progesterone measurement in blood or milk in order to determine the proportion of acyclic females. In addition, some strategies for improving the CR were evaluated.

2. MATERIALS AND METHODS

2.1. Survey

2.1.1. Animals and location

An experiment was performed during a three-year period in 3 dairy herds of crossbred Holstein × Zebu (H×Z) cows and heifers, comprising 6357 animals located in the Western regions of Cuba, in two trials. Selection was done according to the different types of reproductive management used in our country (Table I).

TABLE I. CHARACTERISTICS OF CATTLE HERDS USED IN THE STUDY

Characteristic	Herd I	Herd II	Herd III
Type of farm	Commercial	Commercial	Genetic improvement
Feeding system	Grass, sugar cane, molasses, urea	Grass, sugar cane, molasses, urea	Grass, forage, concentrate
Milking system	Hand	Machine	Machine
N°. of AI technicians	3	3	2
N°. of Bulls	5	10	7

The animals were grazed on improved pasture. Herd I was hand milked and the calves were allowed suckling for milk let down. Farms II and III used machine milking and calves were weaned soon after birth. Mineral mixture and water were available *ad libitum* in all farms. This trial was designed to identify the causes that affect the CR when AI is used in OIS programmes, using progesterone measurement and data management with the artificial insemination database application (AIDA) [7].

2.1.2. Oestrus induction and synchronization (OIS)

This was done using three intramuscular (IM) injections of progesterone (60, 90 and 90 mg) with an interval of 48 hours between each one, followed by an injection of Pregnant Mare Serum Gonadotrophin (PMSG) at a dose of 500 international units (IU) in heifers or 1000 IU in cows.

Oestrus detection was carried out from 06:00 to 10:00 hrs and from 15:00 to 18.00 hrs daily for seven days after the end of treatment. AI was done 8 hours after oestrus was detected, by eight experienced technicians using frozen semen in pellets from 22 bulls from a local AI center. Pregnancy diagnosis was done by rectal palpation 60 to 70 days after AI.

2.1.3. Blood and milk samples

Blood was collected by jugular puncture in heifers and milk was collected from cows at oestrus (day 0) and at 10–12 and 21–22 days after AI. All samples were centrifuged at 2000x g for 15 minutes within 3 hours after collection and plasma or milk whey was stored at –20°C until they were assayed for progesterone using RIA kits supplied by the Joint FAO/IAEA Programme, Vienna.

2.1.4 Statistical Analysis

Data regarding the management and feeding system of the farms, each cow inseminated, the quality of semen used, AI technicians, progesterone results, pregnancy diagnosis and other related factors were entered into the AIDA database and summarized. Further analysis was done using the statistical program SYSTAT for Windows [8] for analysis of variance and the Chi square test.

2.1.5. Further study in one herd with problem of anoestrus

A study was carried out in Herd I in order to know the proportion of cows and heifers which were anoestrous. A total of 389 crossbred (H×Z) females, comprising 127 suckling cows which were more than 60 days after calving and 267 heifers of 24–30 months age and 270–450 kg body weight, which were managed on grass during the dry season were included. In all females two samples of blood (heifers) or milk (cows) were collected at an interval of 9 ± 1 days for analyzing progesterone. Females were considered cyclic when at least one of the samples showed progesterone values greater than 3.18 nmol/L. All lower levels were considered as indicating anoestrus.

Data were subjected to multiple analysis of variance and the proportional test through the SYSTAT program.

2.2. Interventions

2.2.1. Effect of body condition on response to OIS

The aim of this experiment was to evaluate the effect of the BCS on the response to OIS using a different treatment to that adopted in the survey. A group of 594 crossbred heifers (H×Z) subject to AI was used in Herd I under grazing conditions during the dry season.

The treatment for OIS consisted of three injections (IM) of progesterone (60, 90 and 90 mg) with intervals of 48 hours between each treatment, followed by 700 IU of PMSG and 500 µg of an analogue of prostaglandin $F_{2\alpha}$ (Oestrophan, Bioveta, Czech Republic) at the end of the treatment.

Before treatment two blood samples were collected at an interval of 9 ± 1 days in all animals in order to determine cyclic status. After treatment blood samples were collected at the time of AI and at 10–12 days after service. Females with progesterone concentrations >3.18 nmol/L in one of the two samples before treatment were considered cyclic. Females that had a progesterone level <1.59 nmol/L at AI and >3.18 nmol/L 10–12 days after service were also considered cyclic.

Oestrus detection was performed by professional staff and was based on the observation of any sign of homosexual behavior. Observations were carried out every two hours during the day and the first occurrence of cows allowing others to ride them was considered as the onset of oestrus. Insemination was done at fixed times of 48 and 72 hrs after the end of treatment using semen from one fertile bull with the aim of reducing variability due to this factor. The quality of semen was evaluated before its use and certified as being of optimal quality. Pregnancy diagnosis was performed by rectal palpation 60 to 70 days after AI.

2.2.2. Comparison of three methods for OIS

This study was done to determine the effects of three different OIS treatments on the CR of cattle under grazing conditions during the dry season in Herd I. A total of 192 animals were used comprising 85 heifers (24 to 30 months old) and 107 primiparous cows (suckling calves, 80 to 120 days postpartum). All females had a BCS between 2.5–3.5 and an average body weight of 300–450 kg. In order to apply the treatments, females were divided into three groups and treated according to the following main procedure used for OIS in our country.

- Treatment A — Three injections of progesterone (50 mg each, IM) with intervals of 48 hours between them, followed by 500 IU PMSG IM 48 hours after the last injection;
- Treatment B — Three injections of progesterone (60, 90 and, 90 mg, IM) with intervals of 48 hours between them, followed by 500 IU PMSG IM 48 hours after the last injection;
- Treatment C — Three injections of progesterone (60, 90 and 90 mg, IM) with intervals of 48 hours between them, followed by 1 mg of oestradiol benzoate IM 48 hours later.

Detection of oestrus was done twice a day for 7 days after the end of treatment. AI was done according to the AM-PM rule. Pregnancy diagnosis was done 60 to 70 days after AI by rectal palpation.

2.2.3. Statistical Analysis

The effect of treatment on CR was analyzed by Chi square test. Further analysis was done using analysis of variance (GLM by means of SYSTAT for Windows.

3. RESULTS

3.1. Survey

3.1.1. Reproductive performance

The interval from calving to first insemination (CFI) and CR in the three herds are shown in Table II. The mean CFI was 154.3 ± 98.1 days and there was a significant difference between herds (P <0.001).

TABLE II. REPRODUCTIVE PERFORMANCE IN CROSSBRED HOLSTEIN X ZEBU COWS IN THREE HERDS SUBJECTED TO OESTRUS SYNCHRONIZATION AND ARTIFICIAL INSEMINATION

Herds	n	Calving to first insemination (days)	Distribution of intervals (%)			Conception rate (%)
			40–120 days	121–200 days	>200 days	
I	111	240.01 ± 82.00^{c}	3.5	29.5	67	17.6
II	64	143.48 ± 101.84^{a}	56.0	17.0	27	26.6
III	85	101.69 ± 58.39^{b}	81.9	13.5	5.8	12.7

Different superscripts within a column indicate significant differences ($P < 0.001$).

The overall CR was 18%. Thirty seven percent of the treated females in these herds had a BCS below 2.5 points (scale of 1 to 5). These animals represented 48.9% in Herd I, 19.7% in Herd II and 31.4% in Herd III.

Of the batches of semen used, 27% had motility below 30%. Furthermore, 83% of these semen samples were found in herd III. The highest CR was obtained when motility of semen was >30% ($P < 0.05$).

3.1.2. Interpretation of progesterone data

Results of progesterone measurement and their interpretation are shown in Tables III, IV and V. Unfortunately, only 38.6% of the cows were sampled three times according to the AIDA protocol and 3.6% to 23.1% of them had intermediate values. According to our reference data on oestrus determination, we found that 4% of the inseminations were carried out in the luteal phase. When two samples were considered (days 0 and 10–12), the results showed that 33% (213/630) of females were having anovulatory cycles, anoestrus or a short luteal phase, while 11.7% (73/630) were showing intermediate values (<1–3 nmol/L).

TABLE III. INTERPRETATION OF PROGESTERONE DATA BASED ON ONE SAMPLE TAKEN ON DAY OF OESTRUS

	Total	Low values		High values		Intermediate values	
Herds	n	n	%	n	%	n	%
I	581	550	94.6^{a}	14	2.4^{a}	17	3.0^{a}
II	106	80	75.4^{b}	18	7.5^{a}	8	7.5^{b}
III	174	165	94.8^{a}	4	2.8^{b}	5	2.8^{b}
Total	861	795	92.3	36	4.1	30	3.6

Different superscripts within a column indicate significant differences ($P <0.05$).
Low = <1 nmol/L (AI probably during oestrus); High = >3 nmol/L (AI during luteal phase or pregnancy); Intermediate = 1–3 nmol/L (AI done too late or too early, or assay errors).

TABLE IV. INTERPRETATION OF PROGESTERONE DATA BASED ON TWO SAMPLES TAKEN ON DAY OF OESTRUS AND 10–12 DAYS AFTER AI

	Total	Low/High		Low/Low		High/High		High/Low		Intermediate	
Herds	n	n	%	n	%	n	%	n	%	n	%
I	366	164	44.9^{b}	147	40.2^{a}	6	1.6^{a}	3	0.8	46	12.5^{a}
II	72	37	51.3^{a}	12	16.6^{b}	8	11.1^{b}			15	21.0^{b}
III	192	110	57.3^{a}	54	28.1^{b}	2	1.0^{c}	5	2.6	21	11.0^{a}
Total	630	311	49.4	213	33.8	16	2.5	8	1.3	82	13.0

Different superscripts within a column indicate significant differences $P <0.001$.
Low/High: ovulatory cycle; Low/Low: anoestrus, anovulatory cycle or short luteal phase; High/High: AI done in pregnant animal or luteal cyst; High/Low: AI done in luteal phase; Intermediate = 1–3 nmol/L.

TABLE V. INTERPRETATION OF PROGESTERONE DATA BASED ON THREE SAMPLES COLLECTED ON DAY OF OESTRUS, 10–12, AND 21–22 DAYS AFTER AI

	Total	L/H/H, P[1]		L/H/L, NP[2]		L/H/H, NP[3]		H/L/H, NP[4]		Intermediate[5]	
Herds	n	n	%	n	%	n	%	n	%	n	%
I	168	38	22.6[a]	78	46.4[a]	10	5.1[b]			42	25.0[a]
II	45	17	37.8[b]	8	17.8[b]	3	6.7[b]	2	4.4	15	33.3[c]
III	120	27	22.5[a]	72	60.0[c]	1	0.8[c]			20	17.0[b]
Total	333	92	24.6	158	47.4	14	4.2	2	0.7	77	23.1

Different superscript within a column indicate significant differences P >0.001
[1] Low/High/High and Pregnant – AI at correct time and successful; [2] Low/High/Low and non pregnant – AI at correct time but unsuccessful (non-fertilization or early embryonic mortality); [3] Low/High/High and non-pregnant — late embryonic mortality or persistent CL; [4] High/Low/High and non-pregnant – AI during luteal phase; [5] 1–3 nmol/L.

3.1.6. Studies on ovarian activity in a herd with anoestrus problems

As seen in Table VI and VII the ratio of acyclic females is significantly (P <0.001) higher in primiparous cows and in all of the females with poor BCS (<2.5 points).

TABLE VI. PROPORTIONS OF CROSSBRED COWS AND HEIFERS WHICH WERE CYCLIC OR ACYCLIC (ANOESTROUS)

Category	N	Ovarian activity			
		Cyclic		Acyclic	
		n	%	n	%
Heifers	267	147	55.0ad	120	45ae
Primiparous cows	41	7	19.5bf	34	80.5bg
Multiparous cows	76	30	35.5ch	46	64bi
Total	384	184	47.9	200	51.1

Values within the same columm (abc) or row (defghi) indicate significant differences P <0.001.

TABLE VII. EFFECT OF BODY CONDITION SCORE (BCS) ON THE PROPORTION OF CROSSBRED COWS AND HEIFERS WHICH WERE CYCLIC OR ACYCLIC (ANOESTROUS).

BCS	n	Ovarian activity			
		Cyclic		Acyclic	
		n	%	n	%
1–2.4	158	50	27.2[a]	108	72.8[c]
2.5–3.5	226	90	39.8[b]	136	60.2[d]
Total	384	140	36.4	244	63.6

Values within the same column with different superscript indicate significant differences P <0.001.

3.2. Interventions

3.2.1. Effect of BCS on the response to OIS treatment in crossbred heifers under grazing conditions

Poor BCS had a negative and significant effect on the CR (P <0.05, Table VIII). Heifers with a BCS <2.5 showed a higher number of anovulatory cycles and lower CR.

TABLE VIII. EFFECT OF BODY CONDITION SCORE (BCS) ON RESPONSE TO OESTRUS SYNCHRONIZATION TREATMENT AND CONCEPTION RATES IN CROSSBRED HEIFERS UNDER GRAZING CONDITIONS DURING THE DRY SEASON

	BCS	
Characteristics	1–2	2.5–3
Number of heifers	191	403
Heifers cycling before treatment (%)	42.8[a]	60.2[b]
Heifers in oestrus after treatment (%)	87.0[a]	79.0[b]
Heifers with ovulatory cycle (%)	26.9[a]	72.4[b]
Conception rate (%)	8.3[a]	41.3[b]

Values within a row with different superscripts indicate significant differences P <0.05.

3.2.2. Conception rates after three methods of OIS applied to crossbred cattle under grazing conditions

The results are given in in table IX. Analysis of variance showed a significant effect of both treatment and category (heifers or cows) on the CR (P <0.001) and the interaction (Treatment x Category) was also significant (P < 0.001).

TABLE IX. EFFECT OF THE TYPE OF TREATMENT FOR OESTRUS SYNCHRONIZATION ON CONCEPTION RATES IN CROSSBRED CATTLE UNDER GRAZING CONDITIONS

	Total	Heifers	Cows	Conception rate (%)		
Treatments	n	n	n	Heifers	Cows	Overall
A[1]	76	30	46	21.7[a]	56.6[a]	39.4
B[2]	65	30	35	90.0[b]	8.5[b]	46.1
C[3]	51	25	26	0.0	92.3[c]	47.0
Total	192	85	107	34.5	48.2	43.7

Values within the same column with different superscripts indicate significant differences P < 0.001.
[1]Three injections of progesterone (50 mg each, IM) with intervals of 48 hours between them, followed by 500 IU PMSG IM 48 hours after the last injection; [2]Three injections of progesterone (60, 90 and, 90 mg, IM) with intervals of 48 hours between them, followed by 500 IU PMSG IM 48 hours after the last injection; [3]Three injections of progesterone (60, 90 and 90 mg, IM) with intervals of 48 hours between them, followed by 1 mg of oestradiol benzoate IM 48 hours later.

4. DISCUSSION

4.1. Survey

Results obtained in these herds regarding their reproductive performance demonstrate that the long period from calving to first service is related to reproductive management. In the particular case of herd II, it was related to the practice of suckling that takes place in this farm and that causes a lengthening of the anovulatory postpartum period. These findings confirm the results derived from other studies performed under identical management systems [8, 9, 10]. However, the low CR might be associated also with poor semen quality, inappropriate selection of females and the methods of oestrus synchronization.

Previous studies by the authors [11, 12, 13] have shown that poor BCS is related to nutritional anoestrus and due to this physiological condition there is an inappropriate response to controlled breeding programmes [14]. Besides, the finding that some of the doses of semen used were of poor quality suggests that inappropriate handling of semen is an important cause of the low CR in these programmes. The interpretation of the results of progesterone measurement show that a high number of females did not respond to the oestrus synchronization treatment, while others appeared to have been inseminated too late or too early with respect to oestrus. The main reason for not responding to the synchronization treatment is probably due to these animals being acyclic at the beginning of the experiment. This condition was more frequently found in herds I and III.

The study of ovarian activity in a group of females with problems of anoestrus showed that a high number of these were acyclic. Some researchers [15, 16] demonstrated that females under similar physiological conditions with low BCS often show problems of anoestrus. Our results strongly corroborate this fact and highlight the importance of applying appropriate strategies of nutritional supplementation and improved methods of oestrus synchronization in order to increase the CR.

4.2. Interventions

As we already know, the BCS is an expression of the ability to store and use energy in cattle. Previous studies have demonstrated that BCS is closely related to the ability to respond to oestrus synchronization treatments. Our results shown in Table VIII indicate that animals with poor BCS decrease the efficiency of the controlled breeding methods. One of the most important findings was that the mean CR obtained in the group with a BCS of 2.5 to 3.0 (30%) is higher than that previously reported in the survey carried out at the beginning of the experiment. This suggests that with the appropriate selection of females, semen evaluation before inseminations, and with the introduction of some changes in the treatment scheme, the efficiency of AI might be improved under these study conditions. Therefore it is suggested that the nutritional status of females prior to treatment is an important cause of low CR in these controlled breeding programmes. Recent studies have suggested that the above factor is associated with endocrinological changes, with disturbances in growth and development of the ovarian follicle, the corpus luteum, and with some disturbances in embryonic growth and development [16, 17, 18, 19]. The comparative study of different oestrus synchronization methods that have been used showed that treatment B is most efficient in heifers, whereas treatment C is appropriate for cows ($P < 0.001$). The overall means show that the CR was 43.7%, which is higher than those reported by other researchers [20, 21, 22]. It is evident that the CR in these herds can be increased through oestrus synchronization programmes provided that adequate selection of females and careful control of semen quality are performed.

5. GENERAL CONCLUSIONS AND RECOMMENDATIONS

Regarding our findings in the baseline survey, it appears that the poor BCS of selected females as well as the unfavorable quality of semen used in this experiment are the main problems that affect the CR when AI is performed in programmes of controlled breeding. Low BCS associated with anoestrus was found in a high proportion of animals in these herds. The appropriate selection of females and the use of semen of high quality should therefore contribute to overcoming these problems. It is important to improve the nutritional level under these management conditions. It might not be recommended to apply oestrus synchronization treatments to cows or heifers with a BCS below 2.5 points (on a 1–5 scale). The use of the AIDA database to analyze data obtained from progesterone measurements through RIA is a very useful procedure to control and evaluate the quality and efficiency of AI when it is used in controlled breeding programmes in which hormonal treatment is used.

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IDENTIFICATION OF CONSTRAINTS AND IMPLEMENTATION OF CORRECTIVE MEASURES FOR IMPROVING THE EFFICIENCY OF ARTIFICIAL INSEMINATION SERVICES IN DAIRY CATTLE THROUGH THE USE OF PROGESTERONE RIA

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Abstract

IDENTIFICATION OF CONSTRAINTS AND IMPLEMENTATION OF CORRECTIVES MEASURES FOR IMPROVING THE EFFICIENCY OF ARTIFICIAL INSEMINATION SERVICES IN DAIRY CATTLE THROUGH THE USE OF PROGESTERONE RIA

Reproductive parameters were determined in seven dairy farms with intensive system of management in Lima, Peru. Calving to first service interval (CSI) was determined in 552 dairy cows and calving to conception interval (CCI) in 249 cows, in relation to the effects of parity (uniparous and multiparous), breed (Holstein and Brown Swiss), farm and calving season (G1: summer, December to April; G2: winter, May to August; G3: spring, September to November). Data was analyzed by General Linear Model (GLM), and Survival Analysis. The overall CSI (Mean ± SD) was 81.1 ± 35 days (n=552) and CCI was 113 ± 61 days (n=249). Parity and calving season significantly influenced CSI. CSI was 89.9 ± 3.80 days (LS ± SE) in uniparous cows (n = 148) and 81.3 ± 2.65 days in multiparous cows (n = 404; P <0.01), while it was 87.3 ± 2.72, 68.8 ± 2.87 and 100.9 ± 5.72 days for G1 (n = 271); G2 (n = 215) and G.3 (n = 66), respectively (P <0.01). CCI for the same groups were 117.4 ± 4.6, 95.0 ± 6 and 154.6 ± 12 days, respectively, with significant differences between G2 and the other groups (P< 0.01). Parity had no significant influence on CCI. Calving to ovulation interval (COI) was 45 ± 19.9 days (n = 65) and there were significant effects of body condition score at calving, season of calving and farm. The COI was 42.8 ± 2.6 days for cows calving during spring, while it was 23.2 ± 6.1 days for those calving during summer. Cows inseminated during spring had 3.0 more risk probability of wrong insemination. Overall conception rate and first service conception rate were influenced by study period, calving season, service number and origin of semen. Cows calving from September to April had the poorest reproductive indices and should be included in a special breeding management program to improve their performance.

1. INTRODUCTION

Dairy cattle reproductive performance has a great importance due to its influence on overall productivity. Environment has influence on reproductive performance through several mechanisms. In European breeds of dairy cattle, temperature is important, since the zone of comfort temperature for these animals is around 13–18°C [1]. However, in the area around Lima the summer temperature reaches 31°C with 80% of relative humidity. This results in a high temperature-humidity index (THI) [2, 3], causing thermal stress [4], which can result in poor reproductive performance, specifically in breeding animals [5, 6].

Lima is situated at the central coast of the country and reports about reproductive behaviour of dairy cattle indicate that calving intervals are more than 14 months in some farms. Trying to find solutions to this reproductive problem requires identification of the components of the problem, such as the interval from calving to first ovulation, heat detection efficiency, conception rate, and the effects of heat stress in these parameters. It is our purpose to determine the magnitude of these problems and to find the factors that increase the problems in order to suggest, in the future, alternatives to overcome the negative effects of summer in dairy cattle reproduction.

Therefore, the objectives of this survey were to identify the constraints to the efficacy of AI services, determine the influence of calving season, farm, parity, breed and region on reproductive performance, and to study the effect of heat stress on postpartum ovarian activity.

2. MATERIAL AND METHODS

2.1. Location and climate

Lima is situated in the central coast of Peru. The air temperature varies between 12–16°C in winter and 25–30°C in summer. The relative humidity is between 85–95% during the year and annual rainfall is 26.6 to 29.2 mm (Fig.1).

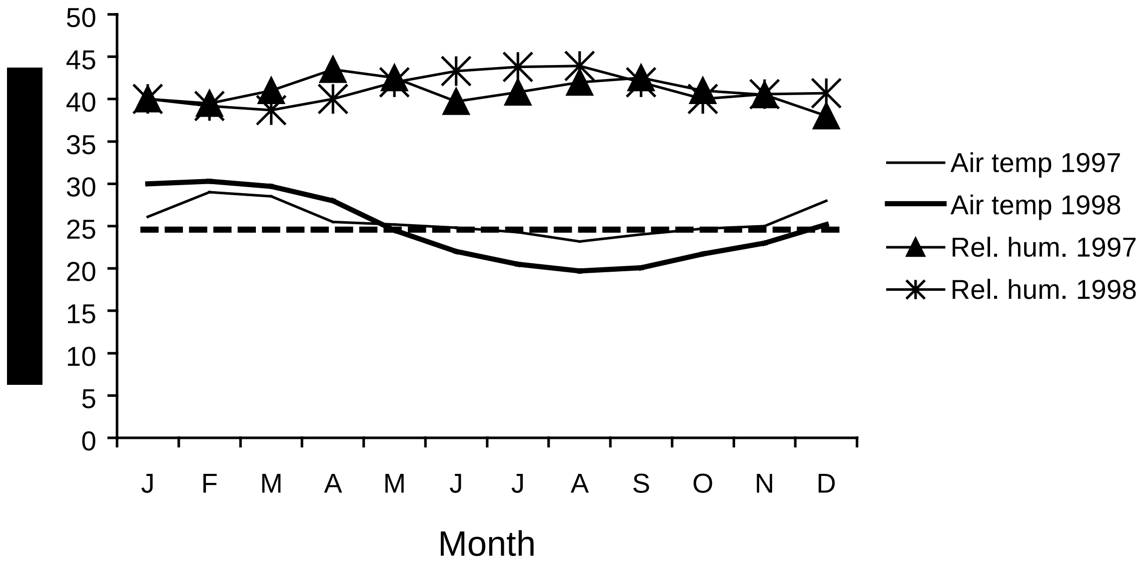

FIG.1. Air temperature and relative humidity in Lima during 1997 and 1998. The dotted line indicates temperature above which cattle are likely to experience heat stress.

2.2. Survey

2.2.1. Farms

Seven farms located in the surroundings of Lima city (3 located 40 km north and 4 located 60 km south) were selected for the survey. The herd size varied from 80 to 800 cows kept in farmyards of 2.5 to 120 ha. The management system was intensive, based on cut forage and concentrates. Milking was done twice a day in most farms, and 3 times a day in a few. Large farms processed their own milk and delivered to the market a variety of dairy products such as yogurt and cheese. Breeding was by artificial insemination (AI), mainly with imported frozen semen. Farms had their own AI technicians. Heat detection was done visually 2–5 times a day.

A total of 557 cows from the seven farms were randomly selected at calving and monitored from their first service till conception in most cases, or at least to the first 2–3 services. Relevant data regarding the farms, AI technicians, inseminated cows, heat expression and insemination details were recorded.

2.2.2. Sample collection

Milk samples were taken on the day of service and two additional samples were collected on days 10–12 and 22–24 after service to assess ovarian activity through progesterone concentration. In case cows returned to heat before collection of the third sample, this was omitted, the cow was re-inseminated, and the sampling procedure reinitiated. Milk samples (5 mL) were collected at milking time in vials containing sodium azide as preservative. Skim milk was separated by centrifugation at 5000 g for 10 minutes and aspiration of the fat portion, and stored at –20°C until assay.

2.2.3. Data management and analysis

Field and laboratory data were entered in the FAO/IAEA Artificial Insemination Database Application (AIDA, developed under MS Access 2.0 for Windows™). The application was also used for basic calculations, simple statistics, and generation of reports. For further analysis, cows were classified by calving number (primiparous and multiparous), by breed of the cows (Holstein and Brown Swiss), and by season of calving (G1: summer, January to April; G2: winter, May to August; and G3: spring, September to December). The information was obtained in two study periods: 1, 1995–1996; and 2, 1997–1998. Data was analyzed by General Linear Model (GLM), where the dependent variables were calving to first service interval (CSI) and calving to conception interval (CCI), and the variation factors were region, farm, breed of the cow, parity and calving season.

The efficiency of the AI service and reproductive herd problems were analyzed by using progesterone values and the result of pregnancy diagnosis by rectal palpation. The progesterone concentration was classified as "L" (low) when it was below 1.25 nmol/L and as "H" (high) when it was above 3.18 nmol/L. This permitted the following four types of interpretations: (1) normal or expected ovarian activity – L H L or L H H; (2) anovulation – L L L; (3) embryonic mortality – L H H and non-pregnant at palpation; and (4) wrong insemination day – H L H, H H L, or L L H.

Analysis was done by Logistic Regression test in univariate and multivariate form. The dependent variable was AI type categorized as Correct (1 and 3 above) or Incorrect (2 and 4 above). The variation factors were CSI, service number, heat to AI interval, swelling of vulva, season of AI (G1, G2, and G3), parity, farm, and location.

Overall conception rate (CR) for 1004 AI services and CR at first services in 639 cows were analyzed by logistic regression in univariate and multivariate form, considering farm location, farm, breed of the cow, part, season of service, CSI, service number, month of AI, interval from heat signs detected by farmer to AI, swelling of vulva, AI technician, and origin of semen (local or imported).

2.3. Technical interventions

Postpartum ovarian activity was monitored in 150 dairy cows through progesterone determination in milk samples collected twice a week from 15 days after calving until confirmation of conception. Cows were grouped by calving season (G1: spring, September – November, and G2: summer, December – March). Body condition score (BCS) was recorded at calving and every 30 days thereafter using the scale 1–5 [7]. Monthly milk production and oestrus behaviour were recorded.

Heat detection efficiency was calculated from heats detected by the farm staff and number of ovulations detected through progesterone analysis. The analysis of reproductive intervals was done by multiple regression analysis, considering calving to ovulation interval (COI), CSI and CCI as dependent variables and parity, season of calving, BCS changes postpartum and farm as independent variables. BCS and milk production were analyzed with repeated measures ANOVA, considering as variation factors the season of calving and parity.

2.4. Radioimmunoassay

Progesterone was determined by a direct solid-phase radioimmunoassay (RIA) using the FAO/IAEA ^{125}I-Progesterone Assay Kit [8]. The intra- and inter assay variation of the internal quality controls were 12.5 and 17.4% for a low pool and 10.2 and 18.8% for a high pool, respectively.

3. RESULTS

3.1. Survey

3.1.1. Reproductive parameters

The CSI in all animals was 81.1 ± 35 days (Mean ± SD, n = 552). The CSI was 89.9 ± 3.80 days (LS ± SE) for uniparous cows and 81.3 ± 2.65 days for multiparous cows (P <0.01). Season of calving had a significant effect (P <0.01) on the results (Table I), but breed of cow and farm did not.

TABLE I. CALVING TO FIRST SERVICE INTERVAL (CSI) AND CALVING TO CONCEPTION INTERVAL (CCI) IN HOLSTEIN COWS DURING DIFFERENT CALVING SEASONS

Calving season	CSI (LS ± SE)	CCI (LS ± SE)
G1 (January – April)	87.3 ± 2.7[b] (n = 271)	117.4 ± 4.6 [b] (n = 152)
G2 (May – August)	68.6 ± 2.8 [a] (n = 215)	95.0 ± 6.7 [a] (n = 74)
G3 (September – December)	100.9 ± 5.7 [b] (n = 66)	154.6 ± 1.2 [b] (n = 23)

[a,b] Means with different superscripts within columns are statistically different (P <0.01)

The overall CCI was 113 ± 61 days (Mean ± SD, n = 249). The factors with significant effects on CCI were calving season (Table I), study period, and CSI (estimate 0.86 ± 0.07). Parity and farm did not affect the CCI.

3.1.2. Milk progesterone determinations

Problems detected in the AI service through the milk sampling protocol were mainly incorrect AI due to poor heat detection (2% of cows bearing active corpora lutea and 9% anoestrus cows). Of the cows submitted to PD (heat not detected after AI) 28.9% were found non-pregnant.

Season of AI and parity had a significant influence on the frequency of incorrect inseminations. Cows inseminated during spring (G3) had 3.0 times higher risk probability of incorrect insemination and multiparous cows had 2.4 times higher risk probability of incorrect insemination (Table II).

TABLE II. PERCENTAGE OF CORRECT AND INCORRECT AI SERVICES IN COWS OF DIFFERENT PARITIES

	Correct AI (%)	Incorrect AI (%)
Parity		
Primiparous	94.8	5.1
Multiparous	87.1	12.8
Season		
Summer	86.5	13.5
Winter	84.0	16.0
Spring	58.3	41.7

3.1.3 Conception rate

First service CR was affected by study period and origin of semen. Cows inseminated during study period 1 (Fig. 2) and those inseminated with imported semen had higher probability of getting pregnant. Overall CR was affected by calving season, study period, (Fig. 2) and service number. Cows inseminated in study period 2 had 2.2 times more probabilities of not getting pregnant, and cows inseminated after fourth AI had higher probability of not getting pregnant.

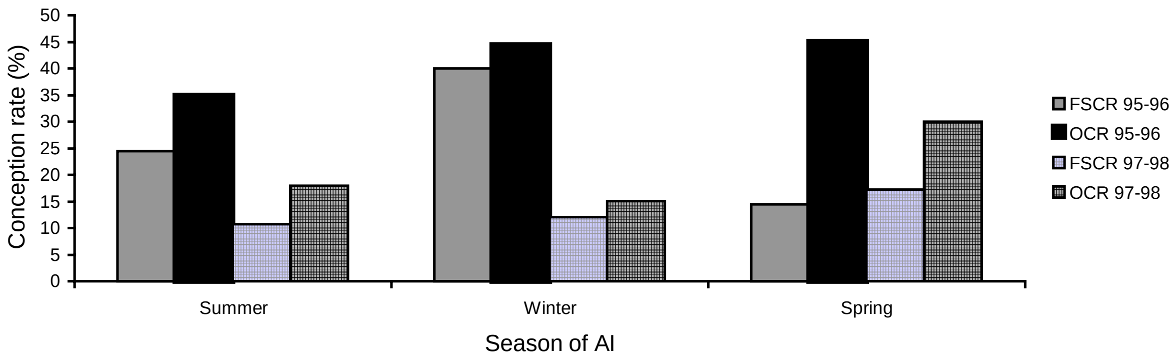

FIG.2. First service and overall conception rates in Holstein cows during different seasons and study periods.

3.2. Intervention trial

The mean (± SD) COI was 45.0 ± 19.9 days (n = 65). There were significant effects of BCS at calving, calving season, and farm. Cows calving during spring had COI of 42.8 ± 2.6 days, while those calving in summer had 23.3 ± 6.1 days (LS ± SE). Heat detection efficiency was determined in 5 farms and was 58, 38, 51, 26 and 0% for farms 1 (n = 29), 2 (n = 18), 3 (n = 18), 4 (n = 11), and 5 (n = 4), respectively.

The mean (± SD) CSI was 99.2 ± 50.1 days (n = 121) for the whole group. This variable was affected by heat detection efficiency, BCS changes, and farm. CCI was 224.6 ± 85 days and was influenced by milk production during first month, farm, COI, and change of BCS between calving to 60 days postpartum. Milk production and BCS changes postpartum were affected by season of calving ($P < 0.01$).

4. DISCUSSION

The number of good quality reports regarding reproductive problems of cows under the existing intensive milk production system in the coastal region of Peru is very limited and therefore, this study initially focused on the evaluation of basic reproductive parameters in order to identify factors responsible for the occurrence of long calving interval (i.e. 15 months).

Theoretically, CSI is affected by the onset of ovarian cyclicity and the efficiency of heat detection. On the other hand, CCI is additionally affected by CR and factors related to it such as semen quality, AI technique, embryonic mortality, and timing of AI [9]. Other factors that can certainly be involved but not considered in this study were those related to infectious diseases. The CSI and CCI found in the present survey were not unduly long when considered as an overall figure. However, when the different variables are individually analyzed as part of the total result, it was possible to identify clear differences between the winter calving group and the others. This situation can be used as an obvious example on how farmers do not usually realize the true and inherent problems in their herds when summarized results are examined.

BCS changes postpartum were significantly affected by season. Cows calving in spring lost more body condition, possibly because of higher milk production and their inability to recover as fast as summer calving cows. Moreover, cows calving during spring had longer anoestrous postpartum periods and delayed onset of ovarian cyclicity, which is reflected by the 43-day COI, which is longer than that reported in the literature for this type of cattle [9]. All this could be related to the postpartum negative energy balance (NEB) observed in high milk yielders [10]. NEB causes hypoglycemia, which increases corticotrophin releasing hormone (CRH), which in turn stimulates ACTH and beta-endorphin release [11], thereby suppressing GnRH pulsatility and LH secretion [1]. The results suggest that the degree of NEB suffered after calving has a greater effect than that of heat stress on the time of first ovulation [10, 12].

Animals that calved in summer had a long CCI. Many of these animals were cycling but had difficulties to get pregnant due to heat stress and low CR [13, 14]. The causes for low CR during heat stress [5] have been attributed to an irregular pattern of oestradiol secretion or elevated uterine temperature that can cause early embryonic mortality [15]. The 40% CR found during winter in our study period 1 (Fig. 2) is considered normal in Peru. However, cows are exposed to THI in excess of 72 in other seasons (temperature of 27–32°C and relative humidity of 80%) [2, 3], resulting in heat stress. In the study period 2, both first service and overall CR were low due to an unusually high environmental temperature in the coast of Peru (El Niño phenomenon, Fig. 1).

A high percentage of AI services were done when cows were not in oestrus. Most of these services were conducted in anoestrus animals and very few (2%) during the luteal phase of cyclic cows, which is similar to other reports [16]. It is quite likely that AI technicians tried to inseminate cows even when they had some doubts whether the animals were in oestrus, in order to get them pregnant before summer. Obviously, such animals did not conceive, resulting in low CR.

It is important to highlight that with the exception of semen source (local semen had a significantly lower CR than imported semen), other factors did not affect CR. The reasons for the low fertility of the local semen is still unclear. However, as semen donors are not tested for infectious reproductive diseases, the possibility exists that such diseases may play an important role in reducing fertility.

It can be concluded that two separate schemes of reproductive management would need to be established. One scheme for cows calving in winter and the other for cows calving in spring and summer. Emphasis should be placed on heat detection efficiency and mechanisms for improving CR. Animals calving in spring should be pregnant as soon as possible, otherwise they may remain open throughout summer. Nutritional management to decrease changes of BCS during the postpartum period in summer may improve productive efficiency, but costs and benefits should be evaluated. Bulls used for semen production should be tested regularly for diseases such as bovine viral diarrhoea that may cause infertility problems. Finally, it is necessary to conduct an economic evaluation of the production system.

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EVALUATION OF A SEASONAL-BREEDING ARTIFICIAL INSEMINATION PROGRAMME IN URUGUAY USING MILK PROGESTERONE RADIOIMMUNOASSAY

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Abstract

EVALUATION OF A SEASONAL-BREEDING ARTIFICIAL INSEMINATION PROGRAMME IN URUGUAY USING MILK PROGESTERONE RADIOIMMUNOASSAY.

To evaluate artificial insemination (AI) services and reproductive efficiency in dairy herds in Uruguay two surveys were conducted in 1995 and 1996. The 1995 survey was done in 10 dairy farms of 3 regions on 696 lactating Holstein cows. The 1996 survey was done in 5 dairy farms in one region and included 768 cows. Precision of oestrus detection and efficiency of AI services were determined by milk progesterone samples taken at days 0, 10 and 23 after breeding and by analysis of the records. In 1995 and 1996, the intervals from calving to first service were 123 and 101 days, and to conception were 158 and 134 days, respectively. Parity, body weight and body condition at calving influenced these parameters, but not body weight or body condition at breeding nor milk production. Accuracy of pregnancy diagnosis by milk progesterone was 70.4%. Heat detection rate was 37.5% and pregnancy rate was 15.6%. In 1997 a second study was done to determine the factors affecting reproductive efficiency in a seasonal breeding AI programme in 328 lactating cows on 3 dairy farms. Milk progesterone measurement revealed that 12.5% of the cows were anoestrous at the beginning of the season and remained so during the trial. The category mostly affected were first-calf heifers (82%). Also, 8.5% of the cows cycling were never reported in heat and this was influenced by farm. Oestrus detection efficiency for cows determined to be cycling by progesterone profiles was evaluated in three periods of 21 days and overall efficiency was 46.9%. Main factor affecting it was farm, with an effect of parity (67.8% in mature cows and 33.2% in first-calf heifers) but no effect of days postpartum. Mean interval from the beginning of the breeding season to first service was 27.4 days, again with a strong farm variation but no effect of parity or days postpartum. In an attempt to improve reproductive efficiency in lactating dairy cows, a treatment protocol was designed, where 414 cows in two herds were synchronised with a combination of gonadotrophin releasing hormone (GnRH) + medroxyprogesterone acetate (MAP) on Day 0 and prostaglandin $F_{2\alpha}$ (PGF) + MAP removal on Day 7, followed by oestrus detection and AI. In Farm A, besides the traditional twice per day (AM/PM) oestrus detection, a third period of observation was included at noon. Progesterone was measured in milk samples to monitor treatment response and to evaluate oestrus detection precision. Interval from PGF to heat was reduced in the farm with three times per day oestrus detection system (6.1 vs. 13.2 days). It was concluded that losses in reproductive efficiency in dairy farms of Uruguay in a seasonal AI programme were mainly due to failure rather than incorrect oestrus detection. More oestrus observation periods improved the response to the synchronisation treatment.

1. INTRODUCTION

Uruguay is mainly an agricultural country and milk production is one of the more important components of the Gross National Product. Dairy farming is pasture-based with utilisation of corn silage, hay and concentrates in varying proportions according to different management systems. Milk production per hectare ranges from 1 000 to 6 000 litres [1]. Breeding is mostly seasonal to take advantage of pasture availability, with one long (autumn/winter) or two short (autumn and spring) breeding seasons in the year. Utilisation of artificial insemination (AI) increased in the last ten years from 15% to more than 50% of the dairy cows and 85% of the dairy heifers [2]. Most of the AI is done as an on-farm activity, with very few AI lines or circuits. Previous surveys [3] have found that the implementation of AI services in dairy farms has been slow due to variable results and lower reproductive efficiency as compared with the use of bulls.

In these seasonal breeding systems, maximising reproductive efficiency is essential because breeding and calving are restricted to a limited period of the year to match milk production with pasture availability [4]. Among individual factors affecting reproductive efficiency, oestrus detection failure is one of the most relevant [5, 6, 7, 8, 9]. Heat detection rate can be defined as the percentage of cows in oestrus that are detected in heat [10] and pregnancy rate is the product of oestrus detection rate and conception rate [11]. Increasing the conception rate is difficult, so improving oestrus detection rate is a more feasible way of improving reproductive efficiency. One possible way is to increase the time devoted to oestrus detection [12] and the other is to implement methods to increase the number of cows in heat in a short period of time, for which a possible tool is oestrus synchronisation [13]. Furthermore, when there is a greater sexual activity in a herd, expression of oestrus symptoms is increased [14, 15] and this could improve oestrus detection rates.

The use of milk progesterone measurement to monitor the major reproductive events such as failure or inappropriate oestrus detection, missed heats and early embryonic mortality, gives more accuracy to the evaluation of the reproductive efficiency of large dairy farms [16].

The objectives of the present studies were:

- To determine the factors involved in the success of the AI services from oestrus detection through conception by means of the analysis of reproductive records and progesterone values obtained from milk samples taken at day 0 (breeding) and day 10 and 23 after breeding;
- To determine ovarian activity of cows at the beginning and during the first 80 days of the breeding period and to evaluate the percentage of missed heats;
- To evaluate the effects of the oestrus detection efficiency obtained in periods of 21 days from the beginning of the breeding period;
- To compare oestrus detection efficiency with two systems of observation for heat in synchronised cows and to evaluate the precision of oestrus detection in cows synchronised in large or small groups.

2. MATERIALS AND METHODS

2.1. Surveys

The study of factors affecting efficiency of AI services started with a survey that was conducted in 1995 and replicated in 1996. Data collected was stored in a database specially designed for this purpose (AIDA, Artificial Insemination Database Application, by Drs Mario García and Oswin Perera, Animal Production & Health Section, IAEA). In 1995 dairy farms from three regions of Uruguay were used and in 1996 the survey was conducted in only one region.

2.2. 1995 survey

2.2.1. Farms, animals and management

Holstein cows (n = 696) were selected from 10 dairy farms in 3 regions of Uruguay as follows: region 1 (South-West) - 1 large farm; region 2 (Centre-South, large dairies with high producing cows)

- 4 large farms; region 3 (North-East, small dairies under extensive husbandry) - 5 small farms. Feeding was based on improved pastures with strategic supplementation of corn silage and concentrates administered in the milking parlour during the winter months. Machine milking was done two times a day in all farms. Heat detection system was based on visual signs (standing to be mounted) and was done twice a day at the time of AM and PM milkings. Breeding was done exclusively by AI and no backup bulls were used**.** Region 1 had only a breeding season from May through September, in region 2 there was a long breeding season from May through March and in region 3 there were two short breeding seasons (winter: May through September and spring: October through December).

The following information was collected: cow identification, calving date, parity, breeding dates, pregnancy diagnosis, monthly milk production, body weight (BW) and body condition score (BCS) at calving and at the day of breeding. BW and BCS at calving and at each service were obtained only in farm A from region 1. Field work started in May, with the beginning of the winter breeding season. All breedable cows were considered, regardless of whether they already had breedings from the previous season or not. Milk sampling started on 10 June and ended in 20 October. Information on all the cows in the ten herds was used for calculation of the reproductive parameters, regardless of whether they were sampled for milk progesterone measurement.

2.3. 1996 survey

Five commercial dairy farms were used from region 2 of the 1995 survey. Cows selected were those with calvings from January until July 1996, as opposed to the 1995 survey where all the cows in the herd were considered. This formed a population of 768 Holstein cows. Reproductive management, feeding and milking were similar to the previous year. Milk sampling started at the beginning of the breeding season on 20 May in all farms and ended in November. Collection of reproductive records continued until the cow was reported pregnant or culled. The farm veterinarian did pregnancy diagnoses every month starting two months after the beginning of the breeding period.

2.4. Milk sampling and progesterone analysis

Milk samples to determine progesterone were obtained on days 0, 10 and 23 after insemination. If the cow came in heat before that time, sampling was reinitiated. Samples were collected in 10 mL plastic vials with a 0.1 g Sodium Azide tablet (Merck) and were submitted to the RIA Laboratory in the Veterinary School of Montevideo, Uruguay. There, samples were centrifuged in a refrigerated centrifuge and the fat-free fraction was stored at –20°C until analysed for progesterone content by a solid phase RIA kit provided by the IAEA, Vienna. Intra-assay CV for samples with values below 1 nmol/L of progesterone was 8.2% and for samples above 1 nmol/L was 9.8%. Inter-assay CV was 11.7% and 4.5% for samples with values below or above 1 nmol/L respectively.

2.5. Interoestrus intervals

The interoestrus intervals were calculated, based on the following grouping, as a parameter of oestrus detection efficiency and to evaluate embryonic mortality (EM) [17]:

1. <17 days (short cycles);
2. 18–24 days (normal oestrous cycle);
3. 25–35 days (EM or incorrect oestrus detection);
4. 36–48 days (EM or one missed oestrus); and
5. >48 days (two missed oestrus or EM or abortions).

2.6. Statistical analysis

The following general linear model for unbalanced data was used to determine the factors affecting the intervals from calving to first service and to conception [18]:

$$INT_{ijklmnopqrs} = \mu + a_i + b_j + c_k + d_l + e_m + f_n + g_o + h_p + i_q + j_r + k_s + \varepsilon_{iijklmnopqrs}$$

where:

INT = Interval to first service or to conception
a = the i^{th} effect of region
b = the effect of farm (A, B, C, D and E);
c = the k^{th} effect of parity (1 and 2);
d = the l^{th} effect of calving type (1, 2, 3 and 4);
e = the m^{th} effect of BW (350, 450, 500, 550, 600, 650 kg);
f = the f^{th} effect of BCS at Calving (1, 2, 3);
g = the o^{th} effect of month of calving (1 to 7);
h = the p^{th} effect of month of breeding (5 to 12);
i = the q^{th} effect of BW at service (450, 500, 550, 600, 650 kg);
j = the r^{th} effect of BCS at service (1, 2, 3);
k = the s^{th} effect of milk production at service (5, 10, 15, 20 25, 30 L);
$\varepsilon_{ijklmnopqrs}$ = aleatory error;

Interactions among variables were tested and comparison among means was done by the LSD method. To analyse overall conception rate at first service, a sinarc conversion of variables was done to transform them as continuous data. Contingency tables were also used to analyse discrete variables.

2.7. Factors affecting reproductive efficiency

2.7.1. Farms, animals and management

A second field study was designed to determine the factors affecting reproductive efficiency and was carried out in 3 commercial dairy farms with more than 100 lactating Holstein cows each. All animals without reproductive disorders that were intended to be inseminated in the breeding season were selected. This formed a population of 328 cows, classified according to the days postpartum (DPP) at the beginning of the breeding season as:

- Cows between 40 and 60 DPP (n = 99) that calved late in the calving season but were beyond the voluntary waiting period of 40 days.
- Cows between 61 and 90 DPP (n = 109) that calved early in the calving season but were still within an adequate postpartum interval to achieve a 12-month calving interval (CI).
- Cows with more than 90 DPP (n = 120) open from the previous breeding season.

Animals were also classified as first-calf heifers (n = 123) and mature cows (n = 205) and the distribution of animals within farms was: farm A = 85, farm B = 91 and farm C = 152.

The study period was of 80 days so that all cows would have a chance to present at least three oestrous cycles.

2.7.2. Methodology

Milk samples were obtained twice a week starting one week previous to the breeding period until the cow was detected in heat and inseminated. When a drop in milk progesterone values to less than 1 nmol/L was preceded and/or followed by at least two samples with values greater than 3 nmol/L it was determined that the cow had an ovulation in that week. This data was matched with the dates of heats detected by visual observation and if a service was done in the same week, it was assumed that the heat was detected or otherwise missed. The progesterone values were also used to differentiate cows cycling or in anoestrus. Handling and processing of milk samples was similar to that in the survey.

2.8. Heat detection efficiency (HDE) and pregnancy rate (PR)

For the purpose of the study, heat detection efficiency (HDE) was defined as the percentage of cows detected in heat and bred from the total cows intended to be bred in periods of 21 days. Pregnancy rate (PR) was defined as the percentage of cows pregnant over the total number of cows intended to be bred in periods of 21 days. These parameters were calculated from the reproductive records.

2.9. Statistical analysis

For the evaluation of factors affecting reproductive efficiency, the interval from the beginning of the breeding period to the first service was analysed by a least squares method for unbalanced data, according to the following linear model [18]:

$y_{i...n} = \mu + a_{i...n} + \varepsilon_{i..n}$

where:

$y_{i...n}$: Interval from the beginning of the breeding period to the first breeding

μ: Overall mean

$a_{i...n}$: matrix vector of the following independent variables:

- Farm
- Parity (first-calf heifer and mature cow)
- Days postpartum

$\varepsilon_{i...n}$: aleatory error

Means comparison was done by LSD at 5% probability. Contingency tables were also used to analyse discrete variables.

2.10. Effect of the frequency of heat detection on a treatment for oestrus synchronisation

In 2 commercial dairy farms with more than 200 lactating dairy cows each, animals more than 40 days after calving and with ovarian activity as determined by presence of a corpus luteum (CL) by rectal palpation, were selected and the following treatment was applied:

Day 0: Injection of 0.25 mg of a gonadotrophin releasing hormone (GnRH) analogue (Gonadorelin, Fertagyl™, Intervet, Boxmeer, Holland) and intravaginal insertion of a polyurethane sponge impregnated with 300 mg of medroxyprogesterone acetate (MAP).

Day 7: Sponge removal and injection of 15 mg of a prostaglandin $F_{2\alpha}$ (PGF) analogue (Luprositol, Prosolvin™, Intervet, Boxmeer, Holland)

In farm A treatment was done in 4 small groups at weekly intervals (n = 42, 49, 40, 40) and 79 other cows in the herd, bred after natural oestrus at the same time, were used as controls. In farm B cows were synchronised in 2 large groups in two consecutive weeks (n = 112 and 131) with 73 cows as controls. Treatments started at the beginning of the breeding season. In farm A oestrus detection was done by the conventional twice a day system when the cows were taken to the milking parlour for the morning and afternoon milking. In farm B an additional observation period of 1.5 hours was done in the late morning.

Milk samples to determine progesterone values were taken at days 0, 7 and 8 (Day 0: beginning of treatment). An additional milk sample was taken at the day of breeding. Processing of samples was similar to that in the survey.

To evaluate the response to the treatment the number of cows inseminated in 30 days was evaluated, so that an oestrous cycle following the induced oestrus could be included. To analyse this, the interval from the PGF injection to insemination was divided in three periods, based on the possible results of treatments as follows:

1. Less than 5 days: cows responding to the treatment
2. 5 to 22 days: cows that came in heat in a period not attributable to the treatment
3. 22 to 30 days: cows responding to the treatment but not detected in heat until the next oestrus.

Pregnancy diagnosis was done by rectal palpation after 45 days in those cows not returning to oestrus.

2.11. Statistical analysis

For the statistical analysis of continuous variables (days from treatment to insemination) a "t" test for paired samples was performed and for discrete variables Chi Square test was done [18].

3. RESULTS

3.1. Survey

3.1.1. Overall reproductive performance

Table I summarise reproductive parameters for the 3 regions in the survey conducted in 1995.

TABLE I. CALVING TO FIRST SERVICE INTERVAL (CSI, MEAN ± SEM), CALVING TO CONCEPTION INTERVAL (CCI, MEAN ± SEM), SERVICES PER CONCEPTION (S/C), FIRST SERVICE CONCEPTION RATE (FSCR) AND PREGNANCY RATE (PR) FROM THE 1995 SURVEY

Region	n[1]	CSI (days)	CCI (days)	S/C	FSCR (%)	PR (%)
1	197	123 ± 6.8[a]	158 ± 7.7[a]	2.4[a]	34[a]	91[a]
2	380	86 ± 1.9[b]	121 ± 3.7[b]	2.7[a]	35[a]	73[b]
3	119	144 ± 7.3[c]	164 ± 10.2[a]	2.2[a]	45[a]	60[c]
Total	696	106	140	2.5	36	77

[1]: n: number of cows
[a, b ,c]: Different letters within columns differ (P <.05)

There were statistical differences in the calving to first service interval (CSI) between the three regions, although calving to conception interval (CCI) was shorter only for region 2. The 1996 survey was done only in farms from region 2 and mean CSI was 101.5 ± 1.9 days (mean ± SEM) and CCI was 132.4 ± 3.2 days. First service conception rate (FSCR) was 40.5% and overall pregnancy rate (PR) 80.5% with 2.4 services per conception (S/C). In both years month of calving affected the CSI and CCI in a similar pattern as shown in Figure 1. The other factors affecting the reproductive parameters are summarised on Table II.

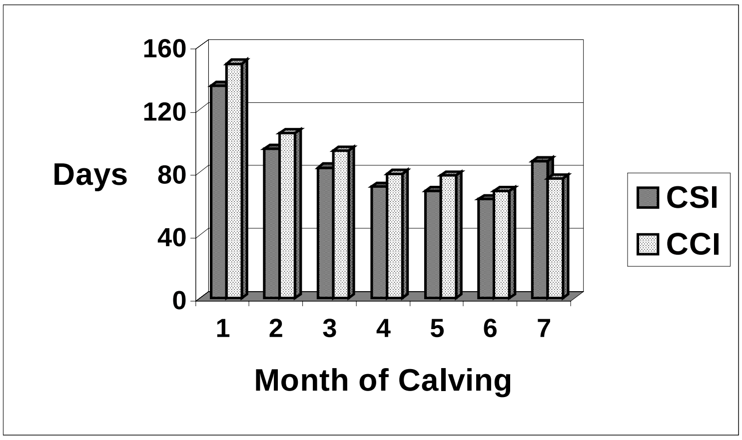

FIG. 1. Average intervals from calving to first service (CSI) and to conception (CCI) according to month of calving.

There was no significant interaction between parity and BW at calving. FSCR was lower for cows with BCS at calving less than 2 (27.8%) than for cows with BCS more than 2 (37.9%) (P >0.1). The BW and BCS at calving did not affect the CCI (P >0.1). Variations in conception rate (CR) were detected also among inseminators in the 1995 survey (34.9% to 48.8%). Significant differences were found among semen donor bulls (P <0.001) with CR ranging from 52% to 15% in those with more than 25 services. There were no effects of BW and BCS at the day of breeding, month of first breeding or milk production on CSI and CCI.

TABLE II. EFFECT OF PARITY, BODY WEIGHT (BW) AND BODY CONDITION SCORE (BCS) AT CALVING ON THE CALVING TO FIRST SERVICE INTERVAL (CSI, MEAN ± SEM) AND CALVING TO CONCEPTION INTERVAL (CCI, MEAN ± SEM)

Parameter	Category	1995		1996	
		CSI (days)	CCI (days)	CSI (days)	CCI (days)
Parity	1	170 ± 11.4^c	200 ± 13.9^a	124 ± 2.9^a	146 ± 3.7^a
	2+	80 ± 8.1^d	114 ± 9.5^b	84 ± 2.2^b	113 ± 3.0^b
Body weight at calving	<500 kg	137 ± 9.9^a	163 ± 11.9^a	118 ± 2.6^a	140 ± 3.7^a
	>500 kg	112 ± 9.7^a	152 ± 11.7^a	85 ± 2.6^b	113 ± 3.2^b
Body condition score at calving	≤2	142 ± 9.8^c	174 ± 10.9^a	113 ± 2.2^a	130 ± 3.3^a
	>2	107 ± 9.9^d	140 ± 11.8^a	88 ± 2.6^b	118 ± 3.2^b

[a, b]: Different letters within columns by year and by parameter differ (P <0.01)
[c, d]: Different letters within columns by year and by parameter differ (P <0.001)

3.1.2. Evaluation of AI services by milk progesterone

In the 1995 survey, a total of 503 breedings with the three milk samples at days 0, 10 and 23 were recorded. Table III summarises the results of milk progesterone as evaluated by the AIDA database application.

TABLE III. PROGESTERONE DATA INTERPRETATION AND DIAGNOSIS BASED ON THREE SAMPLES (n = 503, 1995 SURVEY)

Day 0	Day 10	Day 23	Pregnancy Diagnosis	n	%	Interpretation
LOW	HIGH	HIGH	Positive	196	39	Pregnant
LOW	HIGH	LOW	Negative	79	16	Missed heat
LOW	HIGH	HIGH	Negative	57	11	EM[1]/Abortion
HIGH	HIGH	HIGH	Positive	25	5	AI in pregnancy
*	*	*	Pos/neg	147	29	No diagnosis

[1]EM: Embryo Mortality; *: In doubtful range of values (1–3 nmol/L)

In the 1996 survey, 691 breedings with the 3 samples were analysed and the results are summarised in Table IV.

TABLE IV. PROGESTERONE DATA INTERPRETATION AND DIAGNOSIS BASED ON THREE SAMPLES (n = 691, 1996 SURVEY)

Day 0	Day 10	Day 23	Pregnancy Diagnosis	n	%	Interpretation
LOW	HIGH	HIGH	Positive	338	49	Pregnant
LOW	HIGH	LOW	Negative	88	13	Missed heat
LOW	HIGH	HIGH	Negative	108	15	EM[1]/Abortion
HIGH	HIGH	HIGH	Positive	17	3	AI in pregnancy
*	*	*	Pos/neg	140	20	No diagnosis

[1]EM: Embryo Mortality; *: In doubtful range of values (1-3 nmol/L)

Based on results from the first two samples (days 0 and 10), 4.9% of the cows bred were anoestrous during 1995 and 7.9% during 1996.

3.1.3. Precision of oestrus detection

In the 1995 survey, 22.0% of the 909 breedings were done when milk progesterone values were greater than 1 nmol/L and of this 13.5% were performed when values were greater than 3 nmol/L. In the 1996 survey, 206 (17.0%) of the 1215 milk samples obtained on the day of breeding had progesterone values greater than 1 nmol/L, and 72 (5.9%) of these were above 3 nmol/L. Values between 1 and 3 nmol/L are considered as intermediate, and those greater than 3 nmol/L as indicating CL activity. Accordingly, 11.1% of the cows were bred during the luteal phase, but only 5 cows were bred while pregnant. There was a significant effect of farm on the incidence of incorrect oestrus detection, as presented in Table V.

TABLE V. NUMBER AND PERCENTAGE OF COWS ON DIFFERENT FARMS WITH PROGESTERONE LEVELS ABOVE 3 nmol/L ON THE DAY OF BREEDING

Farm	N	%
A	406	7.4[a]
B	116	8.3[a]
C	179	4.5[a]
D	340	10.3[a]
E	174	30.5[b]
TOTAL	1215	11.1

[a, b]: (Chi Square = 72.6, P <0.05)

3.1.4. Pregnancy estimation (1996)

Based on milk progesterone values at day 23 after breeding, 564 cows were diagnosed pregnant and 397 of these were so confirmed by rectal palpation at day 45+ after breeding, representing 70.4% accuracy for positive diagnoses. For the cows diagnosed pregnant by milk progesterone but found non-pregnant by rectal palpation, the mean interval from the estimated fertile breeding to the following heat was 57 days with a median of 48 days. This long interval was most likely due to late embryonic death.

3.1.5. Evaluation of heat detection efficiency (HDE) and pregnancy rate (PR)

In addition to the information obtained by milk progesterone, reproductive records were analysed to evaluate the heat detection efficiency in periods of 21 days starting at the beginning of the breeding season. A total of 1424 breeding in 6 periods of 21 days were evaluated and the results are presented in Table VI. Overall oestrus detection rate was 37.5%.

TABLE VI. HEAT DETECTION EFFICIENCY (HDE), PREGNANCY RATE (PR) AND FIRST SERVICE CONCEPTION RATE (FSCR) OBTAINED IN 6 PERIODS OF 21 DAYS

PERIOD (dates)	n	HDE (%)	PR (%)	FSCR (%)
1 (5/20–6/10)	380	42.9[a]	17.6[a]	41.1[a]
2 (6/11–7/2)	298	38.3[a]	18.3[a]	47.7[a]
3 (7/3–7/24)	222	36.0[a]	14.3[a]	39.7[a]
4 (7/25–8/15)	206	38.8[a]	14.5[a]	37.4[a]
5 (8/16–9/6)	174	35.1[a]	14.5[a]	42.5[a]
6 (9/7–9/28)	144	34.0[a]	14.6[a]	42.9[a]
OVERALL	1424	37.5	15.6	41.9

[a]: P >0.1

3.1.6. Interoestrus intervals

Figure 2 summarises the intervals between oestrus, which was similar for both years. Nearly 40% of the intervals were greater than the normal range and there were farm differences in both years.

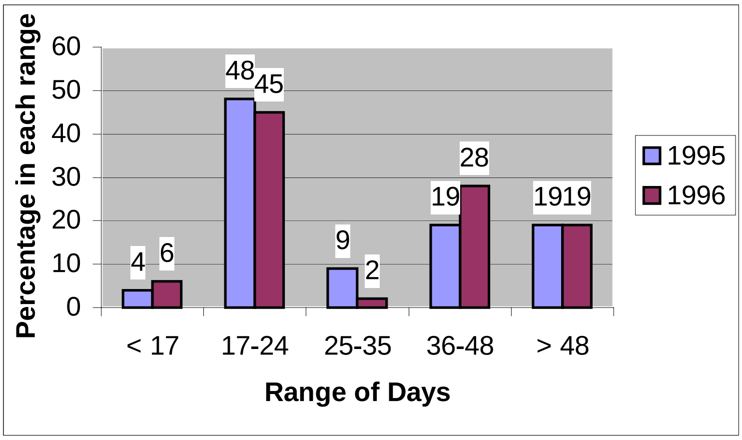

FIG. 2. Percentage of oestrous cycles of different duration

3.2. Factors affecting reproductive efficiency

3.2.1. Incidence of postpartum anoestrus

Cows with more than 4 consecutive samples (2 weeks) with milk progesterone lower than 1 nmol/L were defined as being anoestrous, and comprised 41 (12.5%) of the 328 cows in the selected population. Their distribution according to the different variables analysed is presented in Table VII.

TABLE VII. DISTRIBUTION OF THE PERCENTAGE OF COWS IN ANOESTRUS AT THE BEGINNING OF THE BREEDING PERIOD ACCORDING TO FARM, DAYS POSTPARTUM (DPP) AND PARITY

VARIABLE	CATEGORY	%
FARM	A	30.4[a]
	B	8.9[b]
	C	60.7[c]
DPP	40–60	39.3[a]
	61–90	46.4[a]
	>90	60.7[b]
PARITY	1	82.1[d]
	2+	17.9[e]
TOTAL		100

[a, b, c]: P <0.05; [d, e]:P <0.01

There was a significant effect of farm (P <0.05) on percentage of anoestrous cows. Cows with greater DPP had the highest incidence of anoestrus (60.7%) while no statistical differences were found between 40–60 and 61–90 DPP (P >0.05). Parity was the variable with greatest difference (P <0.01), with 82.1% of the first-calf heifers being anoestrous compared with 17.9% of the mature cows.

3.2.2. Ovarian activity

For the cows that were diagnosed as cycling based on milk progesterone profiles, 491 oestrous cycles were recorded during the experimental period, averaging 1.7 cycles per cow. Of these only 48% were detected by observation while 52% were missed. Twenty-five cows (9.3%) with ovarian activity were never bred. The incidence of cows cycling but not inseminated was influenced by farm (P <0.05), but not by parity or DPP (P >0.05).

TABLE VIII. DISTRIBUTION OF THE PERCENTAGE OF COWS CYCLING BUT NOT DETECTED IN HEAT DURING THE EXPERIMENTAL PERIOD, CLASSIFIED BY FARM, DAYS POSTPARTUM (DPP) AND PARITY

VARIABLE	CATEGORY	%
FARM	A	8.0[a]
	B	20.0[b]
	C	72.0[c]
DPP	40–60	32.0[a]
	61–90	36.0[a]
	>90	32.0[a]
PARITY	1	48.0[a]
	2+	52.0[a]
TOTAL		100

[a, b, c]: Different letters for each variable differ, P <0.05

3.2.3. Factors affecting oestrus detection efficiency

Oestrus detection efficiency was determined in three periods of 21 days from the beginning of the breeding season and the mean values (Table IX) were not significantly different between periods.

TABLE IX. OESTRUS DETECTION EFFICIENCY IN THREE PERIODS OF 21 DAYS FROM THE BEGINNING OF THE BREEDING SEASON

FARM	PERIOD 1		PERIOD 2		PERIOD 3	
	n	%	n	%	n	%
A	21/73	28.8[ac]	26/52	50.0[ac]	21/26	81.8[ad]
B	68/89	76.4[bc]	10/21	47.6[ac]	6/11	54.5[ac]
C	45/125	76.4[bc]	24/80	30.5[ac]	24/55	44.0[ac]
TOTAL	134/287	46.7[c]	60/153	39.2[c]	51/93	54.8[c]

[a, b]: Different letters within columns ($P < 0.05$); [c, d]: Different letters within rows ($P < 0.05$)

There were farm differences within each period ($P < 0.05$) but not between periods for each farm ($P > 0.1$), except for farm A where there was a difference within periods 1 and 2 and period 3 ($P < 0.05$). DPP did not have any influence ($P > 0.1$) on the percentage of cycling cows detected in heat during the experimental period (40–60 = 43.9%; 61–90 = 43.6%; >90 = 51.4%).

Oestrus detection efficiency was higher for mature cows (67.8%) than for first-calf heifers (33.3%) ($P < 0.05$) in the first period of 21 days, however there were no differences between parities within farms.

3.2.4. Interval from the beginning of the breeding period to the first breeding

Mean interval from the beginning of the breeding period to first service was 27.4 days, with significant variations among farms and DPP, but not within parity (Table X).

3.3. Response to oestrus synchronisation

The percentage of cows with luteal levels of milk progesterone at beginning of treatment (day 0) was 57.7%, at PGF injection (day 7) was 69.3% and at 24 hours after PGF injection (day 8) was 8.7%. The number of cows inseminated within 30 days after PGF injection is presented in Table XI.

TABLE X. INTERVAL FROM THE BEGINNING OF THE BREEDING PERIOD TO THE FIRST SERVICE BETWEEN FARMS, DAYS POSTPARTUM AND PARITY (DAYS, MEAN ± SEM)

PARAMETER	CATEGORY	DAYS
FARM	A	32.8 ± 2.3[a]
	B	16.1 ± 2.3[b]
	C	36.1 ± 2.0[a]
DPP	40–60	31.7 ± 2.3[a]
	61–90	30.4 ± 2.1[a]
	>90	23.3 ± 2.1[b]
PARITY	1	30.1 ± 2.2[a]
	2+	25.9 ± 1.5[a]
OVERALL		27.4

[a, b]: Different letters within rows differ (P <0.05)

TABLE XI. NUMBER OF COWS TREATED, PERCENTAGE DETECTED IN HEAT AND INSEMINATED AND INTERVAL FROM END OF TREATMENT TO INSEMINATION IN EACH FARM

PARAMETER	FARM A	FARM B
NUMBER OF COWS	171	243
% INSEMINATED[1]	71 %[a]	80 %[a]
INTERVAL PGF-AI[2]	13.2 ± 1.5[b]	6.1 ± 0.6[a]

[1]: Cows inseminated within 30 days after treatment
[2]: INTERVAL PGF-AI = Prostaglandin injection to AI (days, mean ± SEM)
[a,b]: Different letters within rows differ (P <0.05)

Although not statistically different, a greater percentage of cows were detected in heat and inseminated in farm B. A statistical difference was found in the interval from end of treatment to AI, with 13.2 ±1.5 days for farm A and 6.1 ± 0.6 days for farm B (P <0.05).

When the interval form PGF injection to AI was divided in three periods (Table XII), the percentage of cows inseminated within 5 days after treatment was significantly lower in farm A with a conventional heat detection system (59.8%), compared to farm B where oestrus was detected three times per day (77.7%, P <0.07). While no differences were found in the percentage of cows inseminated between days 5 and 22 after treatment (P >0.05), a greater percentage of cows were inseminated between day 22 and day 30 in farm A (27.9%) than in farm B (7.8%) (P <0.01).

TABLE XII. PERCENTAGE OF COWS INSEMINATED IN THREE PERIODS AFTER TREATMENT

INTERVAL PGF-AI[1]	FARM A	FARM B
<5 days	59.8 %[a]	77.7 %[b]
	(73/122)	(150/193)
5–22 days	12.3 %[c]	14.5 %[c]
	(15/122)	(28/193)
>22 days	27.9 %[d]	7.8 %[e]
	(34/122)	(15/193)

[1]: INTERVAL PGF-AI: Interval from Treatment to AI
[a, b]: P < 0.07, [c]: P > 0.1 [d, e]: P < 0.01 (different letter within rows)

Figure 3 shows the percentage of cows inseminated per day. More than 40% of the cows were bred within 2 days after treatment.

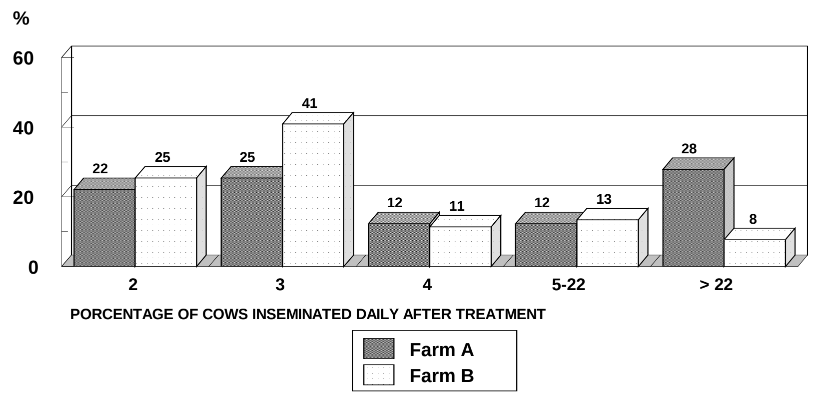

FIG. 3. Percentage of cows inseminated daily after treatment on each farm.

3.4. Precision of oestrus detection

From a total of 467 milk samples analysed from 315 cows synchronised and 152 bred at natural oestrus, only 5.7% had progesterone values higher than 1 nmol/L and of these 2.3% had values above 3 nmol/L. No difference was found between farms or cows bred at a synchronised or natural heat.

First service conception rate in farm A was 56.9% with a difference between synchronised (65.6%) and controls (48.1%) (P = 0.11), while in farm B fertility was very low (30.5%) without differences between synchronised (30.9%) or controls (30.1%). These results are presented in Table XIII.

TABLE XIII. CONCEPTION RATE BY FARM FOR COWS SYNCHRONISED OR INSEMINATED AT NATURAL OESTRUS

GROUP	FARM A	FARM B
SYNCHRONISED	65.6[a]	30.9 [a]
CONTROLS	48.1[b]	30.1 [a]
TOTAL	56.9	30.5

[a, b]: Different letters within columns differ (Chi $Square_1$, P = 0.11)

4. DISCUSSION

4.1. Survey

4.1.1. Reproductive parameters and factors that affect them

Longer intervals from calving to first service (CSI) and to conception (CCI) for region 1 in 1995 were due to a management factor, as spring breeding season in 1994 was eliminated. Thus, cows that calved in winter/spring of 1994 had a longer voluntary waiting period and their first breeding was in May of 1995. There were differences in regions 2 and 3 in the CSI and CCI. Farms of region 3 were small dairies within an extensive region and breeding period was seasonal (winter and spring). This longer interval was due to cows that did not become pregnant in one season and are not bred again until the next season. This also resulted in the lower overall pregnancy rate.

In both years, there was a confounding effect between the month of calving and the interval to the first service and, thus, to conception. This was due to cows with calvings in January and February that had a longer voluntary waiting period because the breeding season started in May. Another confounding effect occurred in Region 3 (1995). This region had two short breeding periods and some cows calving in winter/spring (months July through September) were not bred in the following spring breeding season (October to December), probably due to prolonged postpartum anoestrous. A significant interaction between month of calving and region was detected for these intervals ($P < 0.001$).

Reproductive parameters in both surveys for region 2 were similar, suggesting that year effect was not so marked in farms where grazing was supplemented with hay, silage and concentrates, so feeding did not totally rely on climatic factors. These parameters were similar to those reported for temperate climates [19, 20, 21] or for dry tropics [22]. Mean interval from calving to first service of 106 days (1995) and 102 days (1996) were considerably longer than the goal of 65 days proposed by Morrow [23]. One of the reasons of this longer interval was a prolonged voluntary waiting period due to the seasonal breeding system, a similar effect as reported in New Zealand systems [24]. Figure 1 illustrates this effect and, because the breeding season started on May 20th, cows that calved in January had an interval to first service of 155 days, as opposed to those calving in April that had an interval of 89 days. However with a voluntary waiting period of 40 days the interval never was shorter than 70 days and this can be caused by a prolonged postpartum anoestrous or a poor heat detection, which the methodology used in the survey was not able to differentiate.

Interval to first service was also affected by parity, agreeing with previous reports [25, 26]. Body weight and body condition at calving also affected this parameter, similar to findings by Langley and Sherrington [27]. These authors found that cows with body condition of less than 2.5 at calving had an interval to service of 80 days as opposed to 47 days for animals calving with a body condition greater than 2.5.

4.2. Evaluation of AI services by milk progesterone

Milk progesterone determination is an important diagnostic tool in large herds and has been widely used [16, 28, 29]. In these surveys, cows incorrectly detected in heat ranged between 13.5% (1995) and 11.1% (1996) a greater error than reported by authors that describe percentages lower than 10% [29, 30]. However, errors around 20% have been more commonly found [31, 32, 29]. The error was consistent in different breedings but an important variation among farms was detected as also reported previously [29]. According to Zarco [10] one of the main causes of cows incorrectly detected in heat is the human factor.

Conception rate in cows bred with high progesterone values is zero [28, 33]; however in the 1996 survey, from 206 cows inseminated with progesterone values greater than 1 nmol/L, 44 (21.4%) were reported pregnant from that breeding. From these animals, 71 had progesterone values between 1 and 3 nmol/L and 21 conceived (29.6%). The criteria to decide whether a cow was in oestrus or not was receptivity to be mounted and probably some cows in prooestrus or early metoestrus were reported in heat and inseminated with the results mentioned above.

4.2.1. Pregnancy estimation by milk progesterone and early embryonic death

Progesterone levels obtained on days 22 to 25 after breeding have 100% accuracy in diagnosing non-pregnant cows, but precision of the positive pregnancy diagnosis is around 80% [28, 33, 34, 35]. In the 1996 survey, pregnancy estimation by milk progesterone at day 23 after breeding was only 70.4%, lower than some reports, but closer to the results of Rajamahendran and co-workers [30]. According to these authors, early embryonic mortality is a cause more important than oestrus detection or reproductive failure. Forar and co-workers [24] found an embryonic mortality rate of 10.8% with a period of greater risk between days 31 and 55 of gestation. In this study the mean interval to next heat for cows diagnosed as possibly pregnant by milk progesterone was 57 days, which agrees with the above study.

4.2.2. Evaluation of heat detection efficiency (HDE) and pregnancy rate (PR)

Oestrus detection efficiency was 37.5%, similar to previous reports ranging from 38-43% [20, 21, 22], but lower than some reports of 52% [19] and 74% [36]. With a conception rate of 42% and an oestrus detection efficiency of 37.5%, pregnancy rate was of only 16%. In dairy systems with restricted seasonal breeding periods, this low PR seriously compromises the goal of a 12-month calving interval and partially explains the prolonged period to the first service.

4.2.3. Interoestrus intervals

The analysis of the interoestrus intervals is another way of measuring heat detection efficiency [19, 9]. Evaluated by this method, the efficiency was 58%, which is higher than the 37.5% calculated previously by considering only the first service. A possible cause of this difference is that when only the efficiency of the first breeding is considered, cows in anoestrus were included, which could not be identified under the methodology of the study. According to Esslemont [19] with a good oestrus detection efficiency the ratio between normal interoestrus intervals (17 to 24 days) and abnormal intervals should be 7:1, much higher than the 2.2:1 ratio found in this study. Expressed in another way, from 100 interoestrus intervals, 74% should be within the normal range, higher than the 45% reported here. This analysis confirms the poor oestrus detection efficiency, although it is possible that other factors such as high embryonic mortality due to pathological causes had affected this results.

4.3. Factors affecting reproductive efficiency

4.3.1. Incidence of postpartum anoestrus

Postpartum anoestrus determined by milk progesterone was 12.5% and the population with higher incidence was first-calf heifers (82% of this 12.5%). This is greater than the 6% reported by Bloomfeld and co-workers [37] in cows on pasture in the United Kingdom. The difference was most probably due to a better nutrition in those animals. Similar situations have been described in New Zealand [38, 39] and indirectly relate to lower pasture availability with a higher stocking rate, leading to prolonged postpartum anoestrous periods. According to Macmillan and co-workers [40], non-cycling cows represent the most important infertility problem in New Zealand dairy herds and this condition is a reflection of insufficient energy in the diet after calving and during the breeding season.

Furthermore, in the present study 52% of the ovulations determined by milk progesterone were never observed, similar to previous reports [21, 41]. Also, 8.5% of the cows with normal ovarian activity as determined by milk progesterone were never detected in heat during the 80 days of the experimental period. There was a strong farm effect on this parameter. Adding the percentage of first-calf heifers in anoestrus to those not detected in heat, 37% of the animals in this category were not bred during the season. A similar problem is described in New Zealand [40] in cows in pasture and Fagan and co-workers [42] concluded that the main factor affecting reproductive efficiency was heat detection, but especially in first-calf heifers that are the future of the enterprise.

4.3.2. Factors affecting oestrus detection efficiency (HDE)

Oestrus detection efficiency during the first period of 21 days was 46.7%, greater than the 37.5% found in the survey where all cows were considered independently of their ovarian activity. Differences among periods were not significant. The important differences between farms can be explained by the human factor, as there was only one person in each farm doing the heat detection and this was the same during the three periods. It is interesting to see that the three farms show different situations. In farm A, oestrus detection efficiency was very poor during the first period (28.8%), improving in the second (50 %) and in the third (81.8%). Apparently, as the number of cows to be bred decreased, the efficiency of the detection increased, although this is in contradiction with a previous study [14] which found that a greater number of cows in heat increases sexual activity and thus indirectly increases the possibility of detecting them. Possibly this discrepancy is due to the mechanics of oestrus detection in this system, as it is done when the cows are taken to the milking parlour and, when many cows are in heat at the same time, human failure in identifying and/or recording them all could arise. In farm B, on the contrary, there was high oestrus detection efficiency in the first period (76.4%), decreasing in the second (47.6%). Although there is no firm basis to demonstrate it according to the experimental design, it could be assumed that with good heat detection, all those cows with clear oestrus signs should be detected at their first oestrus. Those ones with weaker symptoms remained for the second period, making the task of detecting them more difficult. Farm C was consistently inefficient in the three periods, agreeing with King and co-workers [43] who concluded that a high percentage of the difficulties in oestrus detection are due to human error or farm management systems rather than a problem with individual animals. The only other factor affecting oestrus detection was parity, again in concordance with previous reports [15].

4.3.3. Interval from the beginning of the breeding period to the first service

If the oestrus detection efficiency were 100% and all the animals were detected during the first 21 days, considering that 4-5% of the cycling animals would be in heat on any one day, the mean interval from the beginning of the breeding season to the first service would be 12 to13 days. Evaluation of this parameter is very useful in systems with seasonal breedings [4, 44] as it allows relating reproductive efficiency with parameters such as intervals from calving to first service, conception and next calving. The interval found in this study was 27.4 days, longer than the intervals of 13 and 15 days reported previously [44, 38], where oestrus detection efficiency was 90%. There was also a marked farm effect on this parameter, which is related to the oestrus detection efficiency of each farm. There were no differences in parity but cows with more than 90 DPP had an interval significantly shorter, again related to a greater heat detection rate in these cows.

In systems with seasonal breeding periods, the prolongation of this interval seriously compromises reproductive efficiency, as it limits the possibilities of having a cow pregnant by the end of the period. Poor oestrus detection rates lengthen the interval to first service and, when conception rate is also low (40%), overall pregnancy by the end of the period would be greatly affected [45].

4.4. Effect of the frequency of heat detection on a treatment for oestrus synchronisation

4.4.1. Progesterone levels

According to progesterone levels at day 0 (beginning of treatment) less than 60% of the cows had luteal values at that time, which is lower than the 71% expected when considering that the dioestrous phase lasts on average 15 days [46]. This difference was probably due to cows in anoestrous that were incorrectly diagnosed as cycling by rectal palpation. Progesterone levels at the time of PGF injection (day 7) were also lower than those reported in studies with cows with ovarian activity determined by rectal palpation at treatment [47]. It is possible then, that a percentage of cows were in anoestrus at the beginning of the treatment. Less than 10% of the cows maintained high milk progesterone levels 24 hours after PGF, a similar response as reported by Twagiamungu and co-workers [48], but lower than the findings of Moreno and co-workers [49] where 100% of the cows regressed the CL after treatment. In the present trial, those animals were not detected in heat within 5 days after treatment as should be expected.

4.4.2. Response to the treatment

Results in Figure 2 show that in the farm with two heat detection periods the response to the treatment was less clustered than in farm B with three detection periods. In the farm with three detection periods a higher percentage of cows were inseminated following treatment. Previous studies have shown that with four periods of oestrus detection 75% of the cows in heat can be detected [50], and with 12 daily observations it is possible to detect 100% of the cows in oestrus [12]. Interval from treatment to insemination was significantly shorter in the farm with more periods of observation. According to previous reports [28] the response to the synchronisation occurs within the 5 following days, with 70-80% of the cows in heat [48]. The lower percentage of cows detected in heat with the twice a day system was due to a greater percentage of undetected heats. Animals that did not responded to the treatment and maintained high milk progesterone levels 24 hours after prostaglandin injection were bred between a 5 and 22 days period, and the percentage was similar in both farms. The greater number of cows detected in heat between days 22 and 30 in farm A corresponded to those responding to the synchronisation treatment but not observed in heat in the first 5 days and were consequently inseminated at the following oestrus.

4.4.3. Precision of oestrus detection and fertility after treatment

The percentage of cows with luteal levels of progesterone when reported in heat were very low (5.7%) and there was no difference in cows with synchronised or natural heats. According to Fogwell and co-workers [52] synchronisation protocols that include an exogenous source of progesterone control oestrus expression better and could be the reason for this low percentage.

In farm A, that had a "normal" conception rate, there was a slight increase in fertility in the group synchronised with a progestin implant. However, larger number of animals would de needed to be able to assess an increase of fertility with this treatment.

5. CONCLUSIONS

The main factor affecting AI services in seasonal breeding programs in Uruguay is poor oestrus detection efficiency. This is mainly due to a human factor, as the greatest differences were detected between farms. The consequence of this is a prolonged interval to first service and to conception (days open). Other factors affecting reproductive efficiency were body weight and body condition at calving and parity. Precision of oestrus detection was not a major problem, although farm differences were also detected in this parameter.

In well-managed dairy herds in Uruguay, postpartum anoestrus is not a major reproductive constraint, although the first-calf heifers are the most susceptible category. Failure to detect cows in oestrus is the major factor affecting reproductive efficiency and this is caused by a human factor, as the greater variation in oestrus detection rate is among farms. In seasonal breeding programs, this oestrus detection failure seriously compromises the reproductive efficiency and the farm management, as more cows would be culled due to reproductive problems.

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EVALUATION OF THE REPRODUCTIVE PERFORMANCE OF CROSSBRED ZEBU CATTLE UNDER ARTIFICIAL INSEMINATION THROUGH THE USE OF PROGESTERONE RIA IN VENEZUELA AND ITS IMPROVEMENT WITH TEMPORARY CALF REMOVAL AND PROGESTERONE IMPLANTS

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Abstract

EVALUATION OF THE REPRODUCTIVE PERFORMANCE OF CROSSBRED ZEBU CATTLE UNDER ARTIFICIAL INSEMINATION THROUGH THE USE OF PROGESTERONE RIA IN VENEZUELA AND ITS IMPROVEMENT WITH TEMPORARY CALF REMOVAL AND PROGESTERONE IMPLANTS.

A survey was carried out to evaluate the reproductive performance of crossbred zebu cattle under artificial insemination (AI). Defatted milk samples were taken for progesterone radioimmunoassay at the moment of AI (day 0), 10 days and 22 days after AI and at manual pregnancy diagnosis. Six farms located in the western region of Venezuela were used in this study and a total of 600 AI were included. The calving to first service interval (CFSI) and the calving to conception interval (CCI) showed no significant differences between the hand milking (suckling) and machine milking (non suckling) systems. However, significant differences ($P < 0.05$) were found among farms within the traditional and hand milking system. The mean (± SEM) CFSI for first calving heifers and for cows with second or higher parity was 141.9 ± 6.9 and 71.8 ± 4.2 days ($P < 0.05$), and the CCI for these two groups was 154 ± 8.9 and 80.8 ± 5.5 days ($P < 0.05$), respectively. Cows calving in the dry season had CFSI and CCI of 115.4 ± 5.2 and 123.8 ± 6.8 days, while for those calving in the rainy season the intervals were 98.3 ± 5.5 and 111.1 ± 7.2 days respectively ($P < 0.05$). Predominantly *Bos indicus* cows had shorter CFSI and CCI ($P < 0.05$) than predominantly *Bos taurus* cows. Overall conception rate, analyzed by Chi-square, showed significant differences due to predominant breed and parity. Correct heat detection, as determined by low progesterone levels at AI, was 95.5% in the best farm and 83.3% in the worst farm. The results of this study identify a postpartum anoestrus problem, especially in the first calf heifers with an important effect of season, breed, farm, and heat detection on the reproductive efficiency of farms under AI. After this survey a study was carried out to evaluate the effectiveness of calf removal for 96 hours compared with treatment using norgestomet implants and PMSG for oestrus induction and fertility in crossbred primiparous acyclic zebu cows which were suckled twice a day. Both treatments had a significant ($P < 0.01$) effect in reducing the CFSI and CCI. Further studies are needed to determine ways of reducing the prolonged anoestrus period in primiparous zebu cows in the tropics.

1. INTRODUCTION

In Venezuela the major population of dual-purpose crossbred cattle is located in the western region around the basin of lake Maracaibo, which accounts for 70% of the milk and 60% of the beef production in the country. Crossbreeding between *Bos indicus* and *Bos taurus* and the use of crossbreed bulls are important tools for success in this production system. These animals are capable of adapting to the environment and show higher reproductive efficiency and increased productivity compared to pure dairy breeds [1, 2, 3]. This particular region has the largest number of farms with artificial insemination (AI) programmes in Venezuela since 1945. However, many farmers are reluctant to use this technique because of low pregnancy rates and the prolonged postpartum anoestrus intervals due to management problems. The low pregnancy rates to AI under these conditions are due to many factors, including sanitary, nutritional, climatic, organizational and socio-economical aspects. Therefore, it is necessary to identify the main constraints of AI and overcome them, in order to allow more farmers to obtain the benefits of this technology and to improve animal productivity in Venezuela. A survey study was therefore designed to evaluate the reproductive performance of crossbred zebu cattle under AI through the use of progesterone radioimmunoassay (RIA), and to identify the main factors which influence reproduction by using this technique in our cattle production system.

A further consideration is that several studies [2, 4, 5] have reported a short period of sexual receptivity in zebu breeds, making oestrus detection more difficult [6, 7]. To improve productivity in crossbred zebu cows it is necessary to achieve a yearly calving interval. To reach this goal, cows must cycle and become pregnant within an average of 90 days postpartum [2, 6, 8, 9]. Suckling or the interaction of the calf with the mother can delay the onset of the oestrus cycle in postpartum cows [2, 10, 11, 12]. It has also been reported that temporary weaning of the calves will shorten the interval to first oestrus and increase pregnancy rates [13, 14, 15, 16, 17, 18]. However, due to several considerations, temporary weaning is not a widespread management practice under tropical grazing conditions.

Attempts to induce a normal oestrous cycle and ovulation in acyclic cows have also involved the use of exogenous progesterone or synthetic progestagen to establish an artificial luteal phase [14, 19]. Methods for administration of progestagens include daily injections, pour-on formulations, oral consumption, intravaginal devices and subcutaneous implants in combination with oestradiol benzoate, oestradiol valerate, Pregnant Mare Serum Gonadotrophin (PMSG) or Gonadotrophin-Releasing Hormone (GnRH) [19, 20, 21]. Factors identified in previous studies which affected the success of progestagen treatments included breed, parity, postpartum interval at the beginning of treatment, season and body condition [4, 19, 22, 23, 24].

After the initial survey a second study was designed to evaluate the effectiveness of calf removal for 96 hours and norgestomet implants in combination with PMSG on oestrus induction and fertility in crossbred acyclic zebu cows which were suckled twice a day, as a method to reduce the prolonged postpartum anoestrus in this type of tropical cattle.

2. MATERIALS AND METHODS

2.1. Initial survey

This study was conducted during one year, in six commercial farms located in the Maracaibo lake basin. The range of mean daily ambient temperature in the region was 26–30°C, mean rainfall was 850–1200 mm/year and relative humidity was 60–70%. The dry season was from December to May and the rainy season from June to November. Animals in the study were crossbred dual purpose (dairy and beef) zebu cows. Breeds represented were Carora (CA), predominantly Brahman (MBR), Brahman × Holstein (MBRH), Brahman × Brown Swiss (MBRP), Holstein × Brahman (MHBR) and Brown Swiss × Brahman (MPBR). All animals were grazing in free pasture-land and were fed concentrates at milking time. Milking was done twice a day (morning and afternoon). In three farms (A, B, C) milking was done by hand and the cows were suckled after each milking by their calf. The other three farms (D, E, F) used machine milking and calves were weaned at 5 days of age.

Ten technicians were registered in the study with educational level ranging from elementary school, high school and professional degree. Semen used in the study was collected from bulls (Brahman, Gyr, Carora, Halton, Holstein, Jersey and Holstein × Brahman breeds) and frozen in medium (0.5 mL) straws with 30 million sperms per dose. All batches were considered of good quality [2, 25, 26].

Milk samples for progesterone measurement were collected at the moment of AI (to determine the efficiency of heat detection), 10 days post AI (to evaluate if oestrus was followed by ovulation and the development of an active corpus luteum), at 22 days post service (for an early non-pregnancy diagnosis) and 45 days post service at time of pregnancy diagnosis by rectal palpation (to confirm the pregnant or non pregnant status of the cows). Samples were taken from the right front quarter in to test tubes previously treated with sodium azide in order to obtain a 0.1% final concentration. The whole milk samples were centrifuged at 3000 RPM for ten minutes and the skim milk kept frozen at –20°C until laboratory processing. Skim milk samples were assayed for progesterone by radioimmunoassay (RIA) using Coat-a-Count RIA Kits (Diagnostic Products Corporation, USA) validated by Plaizier [27]. Progesterone values were classified as low (<1 nmol/L), high (>3 nmol/L) or intermediate (1–3 nmol/L). Inter and intra-assay coefficient of variation were 12.8 ± 1.9 and 3.4 ± 2.7 respectively. All information concerning insemination, cows, farms as well as the progesterone values were recorded using the Artificial Insemination Database Application (AIDA, Joint FAO/IAEA Division, Vienna). A total of 516 cows were recorded in the study.

Data analysis was performed by analysis of variance using the general linear model (GLM) procedure of SAS [28], according to the following linear model: $Y = \mu + P_i + S_j + B_k + MS_l + E_{ijklm}$. Where Y = calving interval to first service and to conception, μ = general mean, P = parity, S = season of year, B = predominant breed, MS = milking system and E_{ijklm} = experimental error. If a significant difference was detected, a least square was used as an indicator of difference among means. Chi-square analysis was used for overall conception rate by parity and predominant breed. The frequency of high, intermediate and low values of progesterone by farm were treated using Fisher's exact test. Data were analyzed at the Zulia University Computer Center.

2.2. Induction of oestrus

This study was conducted in two commercial farms located in the Maracaibo lake basin, Venezuela. The climatic conditions were similar to those in the previous study, but only cows which calved during the rainy season were used in this study. All cows were dual purpose crossbred zebu × Holstein, and were grazing in pasture land of *Panicum maximum* and *Echinocloa polystachia* grass. Milking was performed twice a day and cows were suckled after each milking by their calf. Milk production was measured every 2 weeks. The cyclic condition of the cows was determined by RIA of progesterone in milk samples collected twice a week (Monday and Friday) from 90 to 120 days postpartum. Cows with progesterone values consistently below 1.5 nmol/L were considered acyclic and included in the study. A total of 152 acyclic primiparous cows in moderate to good body condition score (3 and 4 on a scale of 1–5) at 120 days postpartum were allotted to three experimental groups: CR - calf removal for 96 hours (n = 51); NI – 3 mg norgestomet subcutaneous ear implant for 9 days, with an initial intramuscular injection of 5 mg oestradiol valerate and 3mg norgestomet (Crestar, Intervet, Holland), followed by 500 IU of PMSG (Folligon, Intervet, Holland) at the time of implant removal (n = 51); and CG — control group with no treatment (n = 50).

Heat detection was done by visual observation for a period of one hour in the morning and afternoon and also with teaser bulls. Oestrus induction was considered successful if oestrus was detected within 4 weeks of treatment. AI was performed by one technician on each farm according to the international AM/PM rule using frozen semen from four different Brahman bulls.

Milk samples were collected at the moment of AI, to determine the efficiency of heat detection and at 22 days after service for an early non-pregnancy diagnosis. Rectal palpation was done 45 days after service to confirm the pregnant or non-pregnant status of the cows. Samples were processed and assayed as described above. All information concerning insemination, cows, farms as well as the progesterone values were recorded using AIDA.

Statistical analysis was performed by analysis of variance-covariance using Least Square (LS) means for treatment to oestrus, calving to first service and calving to conception intervals. Discrete variables were treatment, farm, AI technician and bull semen. Co-variables were body condition and accumulated milk production to 100 days postpartum. Data were analyzed using the GLM and detected differences between means were compared by LS means of SAS [28]. Frequency tables by Chi-square method of SAS were used to compare oestrus percentage, mean interval to oestrus, first service fertility, pregnancy rate, progesterone level at day of AI and at day 22 after service.

3. RESULTS

3.1. Initial survey

Table I shows the mean interval and standard error of the mean (SEM) for calving to first service interval (CFSI) and calving to conception interval (CCI) in the six farms involved in the study, grouped by milking system. No significant differences were found between milking systems but a significant difference (P <0.05) in these intervals was found only among farms with hand milking.

TABLE I. EFFECT OF FARM UPON THE CALVING TO FIRST SERVICE AND CALVING TO CONCEPTION INTERVALS BY FARM

Milking system and Farm	Interval (mean ± SEM) from calving to			
	First service		Conception	
	n	Days	n	Days
Hand milking				
A	168	$121.0^{a} \pm 7.2$	126	$134.3^{a} \pm 9.1$
B	78	$73.6^{b} \pm 8.4$	52	$72.3^{b} \pm 10.8$
C	106	$155.1^{c} \pm 10.1$	89	$166.1^{c} \pm 12.3$
Machine milking				
D	98	102.7 ± 8.4	68	92.2 ± 10.9
E	42	107.4 ± 11.7	26	122.1 ± 15.9
F	24	81.4 ± 14.9	14	117.9 ± 20.6

[a, b, c] means with different superscripts within a column and variable are different (P <0.05).

In Table II the mean interval and SEM for CFSI and CCI are given by parity and season. A significant difference was found between primiparous cows and second or later parity cows, with longer intervals for the primiparous cows. The influence of the season of calving also significant (P <0.05) with shorter intervals for cows calved during the rainy season.

TABLE II. EFFECT OF PARITY AND SEASON OF CALVING UPON THE CALVING TO FIRST SERVICE AND CALVING TO CONCEPTION INTERVALS

Parity and Season	Interval (mean ± SEM) from calving to			
	First service		Conception	
	n	Days	n	Days
Parity:				
1	120	$141.9^{a} \pm 6.9$	98	$154.2^{c} \pm 8.9$
2 or more	391	$71.8^{b} \pm 4.2$	372	$80.8^{d} \pm 5.5$
Season:				
Dry	250	$115.4^{a} \pm 5.2$	234	123.8 ± 6.8
Rainy	263	$98.3^{b} \pm 5.5$	251	111.1 ± 7.2

[a, b, c, d] means with different superscripts within a column and variable are different (P <0.05).

Crossbred predominantly *Bos indicus* cows with either Holstein (MBRH) or Brown Swiss (MBRP) showed a shorter interval to first service and to conception than crossbred cows with higher *Bos taurus* genetic composition (Table III).

TABLE III. EFFECT OF PREDOMINANT BREED UPON THE CALVING TO FIRST SERVICE AND CONCEPTION INTERVAL

Predominant breed	Interval (mean ± SEM) from calving to					
	First service			Conception		
	n	Days		n	Days	
Carora (CA)	37	112.1 ± 13.5^{ab}		27	136.5 ± 17.4^{ab}	1
Brahman (MBR)	174	101.9 ± 7.8^{ab}		147	115.5 ± 9.4^{ab}	
Brahman-Holstein (MBRH)	95	91.9 ± 9.1^{a}		67	90.5 ± 11.5^{a}	2
Brahman-Brown Swiss (MBRP)	55	94.1 ± 10.6^{ab}	1	34	107.7 ± 13.9^{ab}	
Holstein-Brahman (MHBR)	90	121.9 ± 7.8^{b}	2	57	142.2 ± 10.2^{b}	
Brown Swiss-Brahman (MPBR)	65	$119.4^{c} \pm 9.2^{b}$		43	118.4 ± 12.3^{ab}	

$^{a, b, c}$ means with different superscripts within a column and variable are different (P <0.05).

Chi-square analysis for overall conception rates by parity and predominant breed of the cows is shown in Figure 1. Both factors had significant effects (P <0.05).

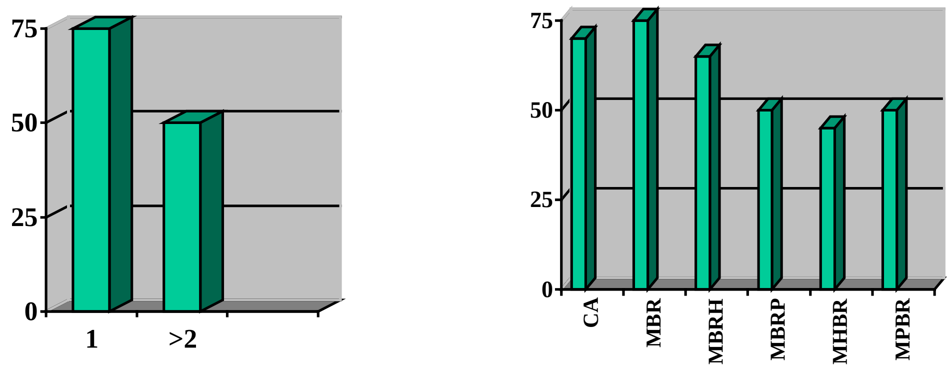

FIG.1. Chi-square analysis for overall conception rate by parity and predominant breed. (P <0.05 ch = 9.26 gl = 1).

The frequency distribution of low, intermediate and high progesterone levels from a total of 600 samples collected the day of AI is shown in Table IV. These results indicate that 92.1% of the cows detected in heat were inseminated when progesterone concentration was within negative range, and therefore at a time other than the luteal phase.

TABLE IV. FREQUENCY (%) OF DIFFERENT PROGESTERONE LEVELS ON THE DAY OF AI BY FARM

Progesterone	Farms					
(nmol/L)	A	B	C	D	E	F
High (<1)	5.2	1.2	2.2	7.5	5.5	0
Intermediate (1–3)	2.6	2.6	3.7	1.8	11.1	9.3
Low (>3)	92.1	92.1	95.0	90.5	83.3	90.3

The progesterone results from three milk samples collected on day 0, day 10 and day 22 of AI in all six farms involved in this study are shown in Table V.

TABLE V. RESULTS OF PROGESTERONE MEASUREMENT IN THREE MILK SAMPLES COLLECTED FROM ARTIFICIALLY INSEMINATED COWS

Day 0	Day 10–12	Day 22–24	Pregnancy Diagnosis	Frequency (n)	%	Interpretation
Low	High	High	Positive	66	52.4	Pregnant
Low	High	Low	Negative	9	7.1	Non pregnant
Low	High	High	Negative	4	3.2	Embryo death
High	High	High	Positive	2	1.6	AI in pregnant cows
Low	Low	Low	Negative	3	2.4	AI in anoestrus cows
Int	Int	Int	Pos/Neg	42	33.3	No interpretation

Low = ≤ 1 nmol/L; High = ≥ 3 nmol/L; Int = intermediate, 1 to 3 nmol/L

3.2. Induction of oestrus

The effects of the three treatments (CR, NI and CG) on percentage of cows showing oestrus, interval to oestrus and conception rate are presented in Table VI. A significantly higher percentage of cows were detected in heat within 4 weeks in CR and NI groups than in the control group, and the interval to oestrus was also shorter in the former groups. The conception rate in those detected in oestrus was similar in all three groups.

TABLE VI. EFFECT OF CALF REMOVAL AND NORGESTOMET TREATMENT ON SUBSEQUENT FERTILITY IN ANOESTROUS PRIMIPAROUS CROSSBRED ZEBU COWS

Treatment	Detected in oestrus		Interval to oestrus	First service conception	
	n	%	(Mean ± SEM, days)	n	%
Calf Removal	26	50.9^a	26.3 ± 6.8^a	16	61.5^a
Norgestomet+PMSG	31	60.7^a	13.8 ± 6.8^a	21	67.7^a
Control	8	16.0^b	61.8 ± 5.9^b	5	62.5^a

[a, b] means with different superscripts within a column are different (P <0.01).

As shown in table VII, a significant difference was found in CFSI and CCI when comparing both the CR and NI groups with the CG, both being longer for cows in the control group. No significant differences were found between the cows implanted with norgestomet and those with calf removal for 96 h.

TABLE VII. INTERVALS FROM CALVING TO FIRST SERVICE AND TO CONCEPTION IN PRIMIPAROUS-CROSSBRED ANOESTRUS ZEBU COWS

Treatment	Interval (mean ± SEM, days) from calving to	
	First service	Conception
Calf Removal	$151.2^a \pm 8.4$	$157.8^a \pm 21.4$
Norgestomet + PMSG	$145.2^a \pm 8.5$	$150.9^a \pm 21.3$
Control	$186.8^b \pm 7.3$	$201.0^b \pm 18.5$

[a, b] means with different superscripts within a column are different (P <0.01).

The progesterone levels on the day of AI were low (<1 nmol/L) in 94.1% of the cows, indicating that AI had been done at a time other than the luteal phase; the conception rate in these cows was 65.9%. Only one out of 9 cows inseminated when progesterone levels were high (>3 nmol/L) became pregnant.

Of the cows with high progesterone levels 22 days after AI (n=117) 81.2% were confirmed as pregnant at rectal palpation, while in those with low progesterone levels (n=36) only one cow was confirmed pregnant. The overall pregnancy rate to AI as determined by rectal palpation was 65.9%.

4. DISCUSSION

4.1. Initial survey

Among the six farms involved in this study farm C showed the longest CFSI and CCI (Table I). This can be attributed to a combination of factors including higher humidity, wet pasture land, more intense sunshine and less shaded areas available for the cows than in the other farms [2, 3, 29]. Farms with milking machines had more uniform and shorter CFSI and CCI. This tendency has been documented in several reports where cows under traditional hand milking with suckling of their calves have longer intervals to first service and to conception. The effect of suckling or even the presence of the calf can block the pulsatile release of LH, delaying the first postpartum ovulation and resumption of normal cycles [3, 30, 31].

The longer CFSI and CCI found in first calving heifers (Table II) is due an anoestrus problem usually caused by low levels of nutrition, heat stress, presence of the calf and failure of heat detection [32, 33, 34]. These results explain the need for better nutritional management of first calving heifers which must be initiated before parturition and kept until the time of recovery of the postpartum ovarian cycle [33, 35, 36]. Preliminary data in our tropical conditions have also shown promising results with the use of bull bio-stimulation and temporary calf removal to shorten the postpartum intervals to first oestrus and to conception. However farmers do not use this management approach as an important tool to increase reproductive efficiency [37, 38]. Cows which calved during the rainy season had shorter CFSI and CCI than those calving during the dry season (Table II), showing the importance of green pasture availability and nutritional level to reduce these intervals [2, 29, 31]. The shorter CFSI and CCI in the Brahman × Holstein cows when compared with those in the Holstein × Brahman and Brown Swiss × Brahman cows indicates the greater degree of adaptation of the former genotype to the hot tropical environment [25, 30, 37, 39].

The effect of parity on overall conception rate is probably due to several factors which are different in the first calving heifers and older cows. Considering the cow breed, the lowest conception rate was observed in the group MHBR which is genotype reported to have lower heat tolerance depressed fertility under high temperature [3, 26, 36].

Measurement of progesterone in milk samples collected at three specific times in relation to AI showed that 92% of the cows detected in heat were inseminated with low progesterone concentration, indicating that accuracy of heat detection was high. Farm C had the best heat detection rate while farm E had the worst, emphasizing the importance of training technicians in this task [32, 34, 40, 41]. The use of teaser bulls with surgical ablation of the dorsal ligament of the penis and caudo-epididymectomy [42], the use of tail paint and increasing the period of visual observation are factors which could improve the efficiency of this particular and very important task in the AI industry.

The results of progesterone measurement in milk show that 52% of the cows inseminated in this study resulted in pregnancy. On the other hand 7% of the cows inseminated when progesterone was low had conception failure and were not detected in oestrus until pregnancy diagnosis. Of the cows that conceived 3% appeared to have suffered late embryonic mortality. Overall, 33.3% of the milk samples had intermediate progesterone values, which could be due to individual variations in oestrous cycle, handling of the samples or variability of assay results. These are in agreement with previously reported data [40, 41], and emphasize the need for rigorous control at each of these stages for accurate interpretation of the results.

4.2. Induction of oestrus

After calving, LH release occurs fairly quickly in suckled beef cows but their levels are too low to trigger ovulation, resulting in long acyclic periods [18, 43]. It has been shown that cows milked twice a day without suckling of their calves have a shorter calving to oestrus interval and better reproductive performance than suckled cows [33, 43]. This is mediated by the capacity of the hypophysis to respond to a GnRH stimulus which is around 10 days postpartum in non suckled cows and 30 days in suckled cows [18, 44, 45]. While some studies have shown that temporary calf removal for 48, 72 and 96 hours can induce oestrus in over 60% of cows, our experience has been that oestrus response is lower than 50%, probably due to genetic and environmental effects [13, 14, 15, 16, 25, 46, 47]. However, even such responses can reduce the losses due to prolonged postpartum anoestrus when compared with the typical proportion of around 16% in continuously suckled cows.

The group treated with norgestomet and PMSG had a significantly higher oestrus rate (60.7%) compared with the control group. During the last few years the use of this regime in acyclic beef cows has become an option for treatment of anoestrus. However, this is still considered costly by many farmers when compared with temporary calf removal. The results of previous studies using hormonal treatments with progestagens vary from normal fertility [47, 49] to lower fertility when compared with natural heat [16, 47, 50]. Results of published reports on the effects of temporary calf removal on reproduction are also not conclusive. While some studies [13, 14, 18, 46, 51] have shown significant improvement in reproduction, others [33, 47] have failed to do so. The effectiveness of temporary calf removal in improving reproduction is influenced by factors such as breed, the period of calf removal, number of days postpartum, body condition of cows at calving and at breeding, and age and parity of the cows [8, 20]. Temporary calf removal has not been reported to have any adverse effect on the calves [52]. In this experiment the fertility at first service was similar for cows with calf removal and the control group. The benefits, however, were in the higher oestrus rate and shorter interval to estrus in cows with temporary calf removal.

The decrease in the CFSI for the norgestomet treated group is similar to data obtained in previous studies [4, 48]. The CCI obtained also corroborates reports of previous workers [53] who found a significant reduction in the interval from treatment to conception, from 37.7 days in a control group to 15.3 days in a treated group.

Temporary calf removal for 96 hr significantly reduced the CFSI and CCI, in a manner similar to that observed Dunn and co-workers with calf removal for 72 hr. On the other hand, calf removal for 48 hr [52] did not result in significant reductions in these intervals.

The progesterone concentration on the day of AI indicated that 6% of the cows were incorrectly inseminated during the luteal phase, which is similar to the proportion reported by other workers [32, 33, 40]. Correct heat detection is an essential requirement for farms using AI. Progesterone determination at the time of AI is therefore an excellent tool to determine the efficiency of heat detection [40]. In some studies, up to 23% of error on heat detection have been reported based on progesterone determination [32, 40], and the fertility obtained at such services have been very low [32, 40, 54].

In this study the apparent pregnancy rate based on high progesterone levels at 22 days post-service was 81%, whereas the actual pregnancy rate determined by rectal palpation at 45 days post-service 66%. This difference of some 15% probably represents late embryonic death. On the other hand, diagnosis of non-pregnant cows at 22 days post-service based on low progesterone levels is highly accurate (95–100%) and the errors if any are mainly due to problems in management of the samples [33, 54]. Determining the non-pregnant status of the cows as early as possible is an important consideration in improving reproductive efficiency of cows, because it allows immediate interventions to be made, either to reinitiate the cycle hormonally, or to observe for the next heat and have the cow mated at the correct time.

5. CONCLUSIONS

Long postpartum anoestrus was identified as the major reproductive problem under the tropical conditions of this study. Parity, breed and farm affected the intervals from calving to first service and to conception. Primiparous heifers and predominantly *Bos taurus* breeds had the longest intervals. Progesterone concentration in the sample collected on the day of AI indicated that heat detection was accurate, with a failure of only 7%, which can be considered good for this farming system. Progesterone levels in all three samples collected from each cow, together with results from rectal palpation, permitted an accurate assessment of the proportions of AI done on anoestrous and pregnant cows, which were also low.

Significant reduction in the intervals to oestrus and conception were obtained in primiparous acyclic crossbred zebu cows with both treatments tested (norgestomet + PMSG and temporary calf removal for 96 h). In this study 94% of the cows were inseminated when progesterone was low, indicating good heat detection. Progesterone levels at 22 days after AI indicated that 81% had probably conceived, but rectal palpation at 45 days after AI showed that pregnancy rate was 66%. The difference of 15% is most likely due to late embryonic death.

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A SENSITIVE PROGESTERONE ENZYME IMMUNOASSAY FOR COW, GOAT AND LLAMA PLASMA USING A MONOCLONAL ANTIBODY AND DANAZOL (17-α-2,4-PREGNADIEN-20-yno (2,3-D) ISOXAZOL-17-ol) AS A DISPLACING AGENT

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Abstract

A SENSITIVE PROGESTERONE ENZYME IMMUNOASSAY FOR COW, GOAT AND LLAMA PLASMA USING A MONOCLONAL ANTIBODY AND DANAZOL (17-α-2,4-PREGNADIEN-20-YNO (2,3-D) ISOXAZOL-17-OL) AS A DISPLACING AGENT.

A sensitive progesterone enzyme immunoassay was developed for cow, goat and llama plasma using a monoclonal antibody and Danazol (17-α-2,4-pregnadien-20-yno (2,3-d) isoxazol-17-ol) as a displacing agent. The microtitration plates were first coated with progesterone 3 (o-carboxy-methyl) oxine: BSA conjugate. The immune reaction was performed by incubating overnight a mixture of 50 µL of plasma and 100 µL of first antibody. After washing, 100 µL of the second antibody (horse radish peroxidase conjugated anti-mouse IgG) were added. The plates were incubated for 1 hour and washed. Immediately the substrate solution was added and finally the reaction stopped and optical density measured. This assay allows accurate determination of progesterone in plasma from several species with good specificity, precision and accuracy, and is suitable for the rapid assessment of luteal function and reproductive status in both clinical and research situations.

1. INTRODUCTION

Progesterone is a good marker for determining the functional status of the corpus luteum (CL). Such measurement is a valuable tool in identifying pregnant or non-pregnant animals, silent heat, lack of cyclicity, cystic follicles, retained CLs and irregular cycles in different species [1–7]. In recent years, considerable attention has been given to Enzyme Immunoassay (EIA) for determining progesterone in different media, due to its low cost and non-radioactive nature. Conversely, the radioimmunoassay (RIA) technique is restricted to laboratories with the capacity to use radioisotopes.

The majority of progesterone in plasma is bound to carrier proteins. In the determination of progesterone concentration by EIA it is necessary to remove these carrier proteins or displace the hormone from them. Thus, earlier methods involved a laborious solvent extraction step, which removes progesterone from its binding proteins. More recently, it has been demonstrated that Danazol (17-α-2,4-pregnadien-20-yno [2,3-d] isoxazol-17-ol), a synthetic steroid, can displace progesterone from plasma proteins in competitive immunoassays [8].

Most solid-phase immunoassays use an antibody linked to the surface of the well. This technique can be expensive, especially when a monoclonal primary antibody is coupled to the polystyrene surface. If the antigen (progesterone 3 [o-carboxy-methyl] oxine: BSA conjugate in this case) is used for the coating of the plates, the amount of antibody required for the assay, and consequently the costs, are considerably reduced.

The objective of the present study was to develop a sensitive, robust and low cost progesterone EIA for cow, goat and llama plasma, using progesterone as blocking agent, a monoclonal anti-progesterone antibody, a horse radish labelled second antibody and Danazol as displacing agent to avoid the extraction procedure.

2. MATERIALS AND METHODS

2.1. Collection and initial processing of plasma samples

All blood samples used for the validation of the assay were collected into heparinized tubes. Plasma was separated by centrifugation and stored at –20°C until analysed.

2.1.1. Reagents

All common reagents were of pure analytical grade (if not specified otherwise, they were purchased from SIGMA, St. Louis, MO, U.S.A.). Assay buffer was prepared by dissolving 0.46 g $NaH_2PO_4 – H_2O$, 1.78 g $NaHPO_4 – 7H_2O$, 8.19 g NaCl and 0.1 g Merthiolate in 1 litre distilled water and adjusting the pH to 7.4 with NaOH. PBS-Tween 20 (PBS-T) was prepared by diluting 2 mL of Tween 20 in 1 litre distilled water. PBS-Tween 20-Danazol (PBS-T-D) was made up by diluting 2 mg of Danazol in 1 litre of PBS-T. The coating solution was progesterone 3 (o-carboxy-methyl) oxine: BSA conjugate (Sigma P4778, 300 µg/100 mL assay buffer) and the blocking solution was bovine albumin (Sigma A7030, 3.0 g/100 mL assay buffer). Washing buffer was prepared by dissolving 9.0 g Sodium Chloride and 0.5 mL Tween 20 in 1 litre of distilled water.

The first antibody was a mouse monoclonal antibody to progesterone (Serotec MCA 2008, NovaKemi AB, Sweden). The original solution (1mg/mL) was diluted 1:10 with assay PBS-T and stored in aliquots of 100 µL at –20°C. Before use, the solution was diluted to 1:100 with PBS-T and further diluted to 1:8000 in PBS-T-D. In the case of llama samples, the first antibody was diluted to 1:12.000 in PBS-T-D.

The second antibody was Anti-Mouse IgG, raised in goat and conjugated to Horse Radish Peroxidase (Sigma A4416). The original solution was diluted 1:10 with assay PBS-T and stored in aliquots of 100 µL at –20°C in glass tubes. Before use, the solution was further diluted to 1:1000 with PBS-T. Stock solution of progesterone standard containing 1000 ng/mL alcohol, was further diluted 1:10 in alcohol (100 ng/mL = 318 nmol/L). Two mL of standard 318 nmol/L were evaporated and re-dissolved in 2 mL of plasma with zero levels of progesterone from cow, goat or llama. Thereafter it was serially diluted with the appropriate zero plasma to obtain an standard curve ranging from 0.62 to 159 nmol/L.

For each species, zero plasma was prepared from a pool of plasmas containing low progesterone concentrations. Plasmas were extracted twice by diluting them 4:1 with diethyl-ether (Merck, Argentina). The aqueous phase was frozen at –25°C and the solvent discarded. Any ether remaining in the plasma was finally evaporated under nitrogen stream at 37°C. Extracted plasmas were subsequently checked for progesterone concentrations using a commercial kit (CAC, DPC, Los Angeles, Ca.). In all cases, non-detectable concentrations were recorded.

2.1.2. Preparation of microtiterplates

Rigid 96 well microplates for EIA (Cliniplate®, Labsystems, Finland) were incubated overnight at 4ºC with 100 µL of coating solution. After washing the plate 3 times with washing buffer, 200 µL of the blocking solution were added into each well and the plates incubated over night at 4°C. Thereafter, the plates were washed 3 times with washing buffer, dried, covered with plastic film and stored in the freezer until used.

2.1.3. Progesterone EIA procedure

Standards, controls and unknowns (50 µL) were dispensed into appropriate wells followed by 100 µL of the first antibody, except for the blanks, where 150 µL of zero plasma were pipetted, and incubated overnight at 4ºC. The plates were washed 3 times with washing buffer and 100 µL of the second antibody were dispensed into each well. After one hour incubation at room temperature, the plates were washed 3 times and 100 µL/well of the chromogen (OPD, SigmaFast, Sigma P9187) were added. The plates were kept for 20–30 min in darkness until colour developed. The reaction was stopped with 50 µL of a 12 % solution of sulphuric acid. Absorbance was measured in an automatic plate reader (Multiskan EX, Labsystem) using a 492 nm filter.

2.1.4. Biological samples

Blood samples from 38 goats were collected between day 22 and 24 after mating. Pregnancy was assessed by ultrasound and confirmed retrospectively by the observation of a live kid. Intravaginal devices containing progesterone (2.0 g and 1.0 g) were inserted in 3 ovariectomised cows and in 3 llamas, respectively. Blood samples were collected daily, from the day before insertion until day 10 after insertion. All blood samples were collected into heparinised tubes.

3. RESULTS

3.1. Titration of anti-progesterone antibody and enzyme labelled antibody

For routine use in the EIA, optimum dilution of the first and second antibody were set at 1:8 000 and 1:1 000 except for the llama system where the first antibody was used at a dilution of 1:12 000 in order to increase the sensitivity of the assay. Fig. 1 displays the influence of increasing the dilution of the first antibody on the sensitivity of the assay.

3.1.1. Assay validation

The precision profiles of six standard curves per species were utilised for the validation of the assay.

3.1.2. Parallelism

Fig. 1 shows the standard curves and the parallel displacement curves produced by serial dilutions of goat (a), cow (b) and llama (c) plasma containing high progesterone concentrations. The dilution was performed using zero plasma from the respective species.

3.1.3. Assay sensitivity

The sensitivity of the assay, defined as the intercept of maximal binding –2SD, was 0.4 nmol/L for goat and cow plasma, and 0.8 nmol/L for llama plasma. The ED-50 was approximately 5 nmol/L; 20 nmol/L and 60 nmol/L in standard curves of goat, cow and llama plasma, respectively.

3.1.4. Intra-assay coefficient of variation

When goat plasma was used in the standard curve, the intra-assay coefficient of variation was around 12% at 0.6 nmol/L and below 5% for concentrations from 1.2 up to 159 nmol/L. When cow plasma was used, the respective value was below 10% for concentrations ranging from 0.6 to 159 nmol/L. For llama plasma, the intra-assay coefficient of variation was around 13% at 1.2 nmol/L and remained below 12 % for concentrations up to 159 nmol/L.

3.1.5. Inter assay coefficient of variation

Zero plasma from goat, cow and llama was supplemented with progesterone in order to obtain a “low control”, containing about 5 nmol/L and a “high control” containing about 80 nmol/L. The controls were assayed in duplicate for each of the standard curves. The inter-assay coefficient of variation was 5 and 7%; 7 and 9% and 7 and 11% for the low and high control in goat, cow and llama plasma.

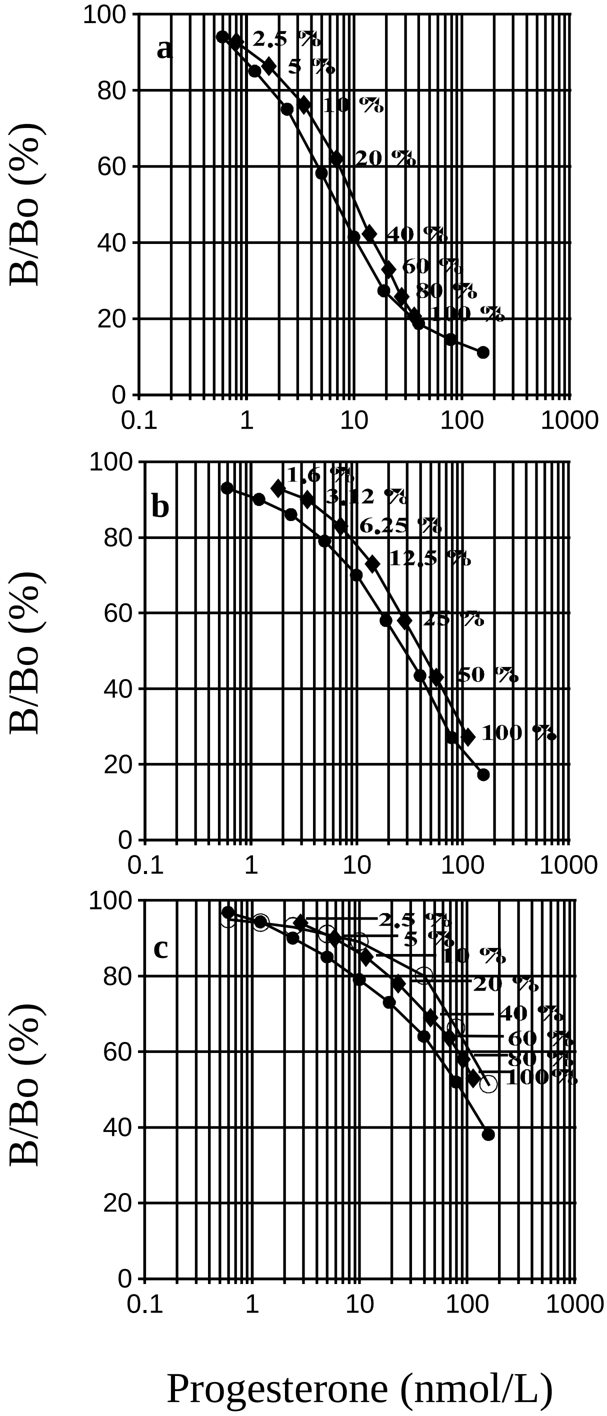

FIG.1. Standard curves (●) and the parallel displacement curve (◆) produced by serial dilutions of cow (a) goat (b) and llama (c) plasma containing high progesterone concentrations. In 1c the influence of increasing the dilution of the first antibody (○ = 1:8.000; ● = 1:12.000) on the sensitivity of the assay is displayed.

3.1.6. Clinical validation of the assay

Based on the ultrasonographical examination and the reproductive outcome, goats were classified as pregnant or non-pregnant at the time blood was collected. Mean (± sem) plasma progesterone concentration in non-pregnant animals was 3.8 ± 1.2 nmol/L (n = 25), while in pregnant animals, the value was 32.2 ± 3.2 nmol/L (n = 13).

The mean progesterone profile of 3 ovariectomised cows (a) and 3 llamas (b) receiving exogenous progesterone is shown in Fig. 2.

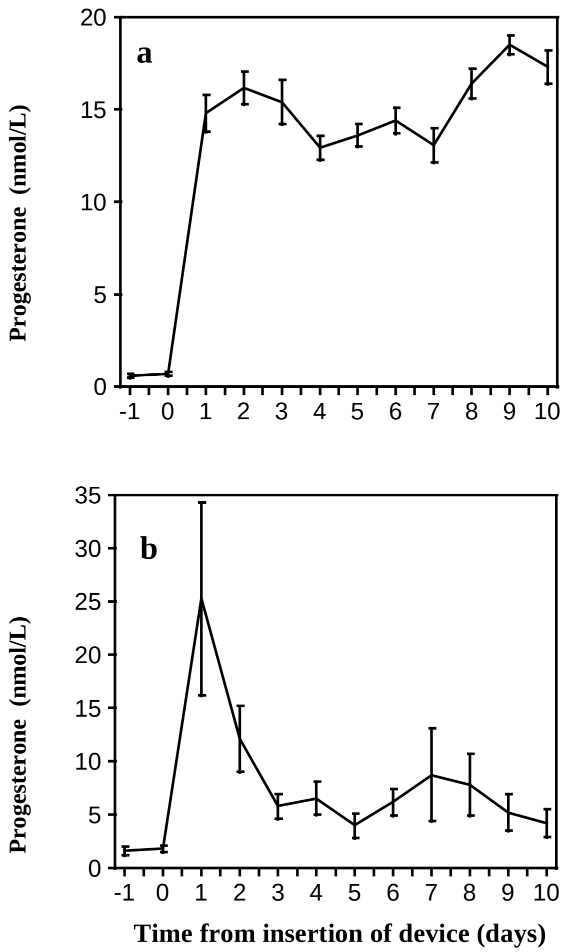

FIG. 2. Mean (± sem) progesterone secretory profile in 3 ovariectomised cows (a) and 3 llamas (b) after the insertion of a progesterone releasing device on day 0.

4. DISCUSSION

In the present study, the need for extraction of progesterone from its transport proteins was circumvented by using a displacing agent Danazol (17-α-2,4-pregnadien-20-yno (2, 3-d) isoxazol-17-ol). This represents an important advantage of the method from the practical point of view since time and money is saved. Moreover, the fact that the plates are coated with the antigen (progesterone) instead of the more expensive antibody (monoclonal in this case) as is the usual practice, represents an additional advantage since the cost of the assay is decreased considerably.

The observation that plasma from the species to be tested must be used in the standard curve, suggests that matrix effects occurred, which may have been amplified by species differences in plasma proteins. This phenomenon might also account for the lower sensitivity registered by the assay in the llama system. This was also reflected in the ED-50 registered in the different species. It could be considered as relatively high in the case of the llama system, giving further support to the hypothesis that the composition of llama plasma (or the affinity of its transport proteins) may interfere with the assay. This was also reflected in the slope of the standard curve in llama system, which was significantly lower than in the cow and goat system. Acceptable sensitivity and variation at low concentrations as well as low non-specific binding were obtained for plasma from all 3 species.

It may be concluded that the progesterone EIA for cow, goat and llama plasma, using progesterone as blocking agent, a monoclonal anti-progesterone antibody, a horseradish labelled second antibody and Danazol as displacing agent, is robust and cheaper than other conventional immunoassays. Thus, when commercial kits are used, the cost per sample (run in duplicate and considering standard curve and controls) varies between US$ 3 and 5, depending on the kit. Using the EIA described in this paper, the cost may be reduced to less than US$ 1 per sample. This assay allows accurate determination of progesterone in plasma from several species with good specificity, precision and accuracy, and is suitable for the rapid assessment of luteal function and reproductive status in both clinical and research situations.

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NEW PERSPECTIVES AND OPPORTUNITIES FOR IMPROVING REPRODUCTION IN DUAL PURPOSE CATTLE

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Abstract

NEW PERSPECTIVES AND OPPORTUNITIES FOR IMPROVING REPRODUCTION IN DUAL PURPOSE CATTLE.

Traditionally, cattle raised under the tropical conditions of the lowland tropics have been dedicated to beef production. However, in the last years, considerable interest has been given to milk production. Hence, the logical step has been the development of dual purpose cattle, thus avoiding losing the income generated by the sale of beef. This concept is particularly important, as the introduction of specialised dairy breeds has mainly proved an unsatisfactory solution to increase milk production in the area. Dual purpose systems, however, have been limited due to poor reproductive performance and are facing considerable dilemmas such as: a) degree of heterosis needed in the cattle for optimal production and adaptability to the tropics; b) suitable management systems, i.e. dual purpose cattle where the main income is beef and milk production is just an added bonus, or conversely, where milk is the main income for the farmer; c) insufficient economical resources of the farmer, those with only subsistence levels as opposed to farmers with certain investment capacity; d) choice of breeding systems, artificial insemination versus natural mating; e) implementation of feeding practices, capacity to implement strategic supplementation in contrast to cattle raised almost exclusively at pasture; and f) adequate marketing of dairy products in the tropics. These considerations related to the strength and weaknesses of dual systems are discussed in this review.

1. INTRODUCTION

The cattle population of Latin America accounts for about one-third of the total number of bovines found in the tropics worldwide, which was estimated to be about 740 million [1]. Reviews on the performance of cattle raised under tropical conditions have shown that these animals fall far short of their maximum potential. For example, Sere and Vaccaro [2] found that the average milk production for the Latin American region was 1070 kg per lactation, compared to 3586 kg for Europe and 5259 kg for the United States.

Management systems can obviously be considered responsible for the vast differences. However, for Australia, where conditions are probably similar to those of Latin America, average milk production was 2873 kg per lactation. Chicco and Schultz [3] estimated that only 25% of the milk and 50% of the beef requirements for the region are produced in tropical areas. Studies by the FAO conducted from 1984 to 1989 have indicated that developing countries account for only about 30% of world beef production.

On the other hand, it has been customary to transfer technology developed in industrialised countries to the developing world, with the aim of improving traditional husbandry procedures for livestock management. This approach has been reasonably successful with cattle raised in environments similar to those for which the technology was created. In Latin America for example, this is particularly true for Holstein cattle living in temperate zones. Nonetheless, it is estimated that almost two-thirds of cattle raised in this region is concentrated in the lowland areas, where the native breeds of cattle, predominantly a mixture of European and Indian crossbreeds, are raised under harsh environmental conditions. The economical success of pure European breeds in such environments has been seriously questioned [4].

This situation has created the need to adapt imported technologies to the unique local conditions prevailing in the Latin American lowland tropics. However, few research efforts have focused on answering questions created by using systems developed in other areas of the world. Also, available results have been generally difficult to interpret due to he diversity of conditions where the animals are raised. Moreover, few studies have been concerned with the evaluation of existing methodologies that are perhaps more suitable for the region than sophisticated techniques created elsewhere.

Consequently, appropriate technologies will have to be created to maximise the reproductive potential of these animals. This is not an easy task, as there is a need to identify the constraints to optimal productivity inherent in each specific management system before any effort is made to improve cattle production. Also, constraints need to be identified wherever research is needed to overcome the obstacles.

It is certainly not easy to conduct the type of applied research needed to address these problems. It calls for a sound understanding of the target farming system, requires an interdisciplinary approach and must judiciously combine empirical on-farm studies with advanced laboratory techniques. The ultimate aim should be to develop cost-effective interventions based largely on indigenous resources. The objective of this paper is to analyse some of the common constraints that jeopardise the reproductive performance of dual-purpose cattle raised under tropical conditions and to formulate general recommendations that might be suitable for the region.

2. REPRODUCTIVE PERFORMANCE

2.1. Degree of heterosis needed in cattle for optimal production and adaptability to the tropics

There is substantial evidence that certain breeds, particularly of the *Bos taurus* type, tend to have their age at first calving much earlier than *Bos indicus* breeds. However, the interpretation of the data rather than the nature of the data itself may account for some variation. Table I adapted from the review of Galina and Arthur, [5] shows that age at first calving usually occurred about 40 months for the *Bos indicus* breeds and 32 months for the *Bos taurus*. Taking these data in isolation one would argue in favour of the benefits to be gained by the rearing of European breeds in the tropics, as they calve earlier in the herd for the first time, and subsequently have a similar calving interval to *Bos indicus* breeds. However, this is most likely the wrong conclusion as it ignores other important considerations. For instance, European cattle are usually allocated in the best pastures of the farm, putting Indian breeds in disadvantage. These native animals are judged on the basis of their natural resistance to the environment, to be capable of doing well on poor pastures. Moreover, few studies published on the reproductive performance of a particular farm provide enough information for account to be taken of the quality of the management system, or nutritional practices, nor do they detail variables, such as the percentage of animals being culled, stocking rates, or breeding methods; i.e. artificial insemination as opposed to natural mating. This is understandable when we consider that most of these studies are retrospective, making it difficult to ascertain the conditions of these animals years before the onset of the study.

TABLE I. AVERAGE AGE AT FIRST CALVING IN MONTHS IN DIFFERENT CATTLE BREEDS UNDER TROPICAL CONDITIONS

Breeds	No. of Studies	Mean	Standard Deviation	Standard Error	Minimum	Maximum	95% Confidence Interval
Bos Taurus	86	32.2	5.9	1.9	26.0	47.2	27.0–37.5
Bos Indicus	65	44.4	6.4	2.1	33.6	54.8	38.7–50.1

Productivity of cattle in the tropics is poor; it has been observed that under the present conditions, about 30% of the replacement heifers will not reach weaning or branding. While another 20% will not even calve for the first time prior to their release from the herd [6]. This situation gets worse when cattle of European origin are introduced into the tropics. Vaccaro and co-workers [4] monitored the production and life expectancy of imported Holsteins to Venezuela; 81.3% of the calves born from these animals died before one year of age, and the number of calvings recorded for these animals during their lifetime in the herd was only 1.2.

As a consequence, crossbreeding of *Bos taurus* and *Bos indicus* breeds has emerged as a useful alternative for cattle production in the tropics. Nevertheless, a definite methodology has yet to be established. For example, Plasse [7] demonstrated that upgrading Criollo cattle to Zebus has been successful only up to the second generation. Cunningham and Syrstad [8] in an excellent review on crossbreeding techniques in *Bos taurus* and *Bos indicus* cattle raised for milk production, concluded that in the crossbreeding systems predictions depend on the estimated level of additive and heterosis effects. The accuracy of the prediction will depend on the validity of these estimates in the environment in which the system was implemented. All this means that crossbreeding with a particular combination of breeds in one location may not be appropriate to another environmental situation. Similarly, Galina and Arthur [5] indicated that when crossbreeding *Bos taurus* and *Bos indicus*, the performance of the resulting crosses was in accordance with the amount of the taurine genotype present in the animals. However, further crossbreeding of the resultant F1 gives unpredictable results. In this same study, it was found that interspecies crossbreeding did not favour reproductive performance and unspecified crossbred cattle showed the worst accomplishment. Similar findings were reported [9] in a large comparative study of dairy, beef and dual-purpose cattle. The worst performance was obtained in the latter type (Table II). In general, it is agreed that for adequate performance and endurance, it is desirable to have at least 25% of *Bos indicus* blood in the crossbred animal. However, to add to the controversy, a recent study containing large numbers of animals [10] clearly demonstrated that the resulting crossbred between *Bos taurus* and *Bos indicus* above ½ of heterosis increased the frequencies of disposals due to health, accident and other causes, reinforcing earlier findings of this group [11], indicating that animals raised under low or high management in Brazil, the F1 crosses outperformed the combinations from ¼, until almost pure-breds. These long-term studies strongly indicate the need of establishing F1 programmes for milk and meat production in the tropics. Nonetheless, more studies are necessary as the disparity in information could be due among other things to the effect of heat stress, this in turn affecting the cascade of reproductive hormones. However, this important concept requires investigation as some breeds are better adapted than others to tropical conditions are. More information is needed on the environmental causes of poor reproductive performance of the various genotypes raised under tropical conditions, as to be able to gain an insight into the physiological mechanisms responsible for these effects.

2.2. Suitable management systems

Dual-purpose cattle raised under the conditions of the lowland tropics are exposed to an array of management systems, which affect their performance. In a study carried out in Mexico [12] it was found that cows predominantly of European breeds housed in comfortable environments tended to calve in the cool winter months, whereas similar type cattle, maintained in poor conditions calved in the hot spring-summer season where natural pastures are available. Similarly, a comparison of the onset of ovarian activity in Holstein and Jamaica Hope raised in the tropics [13] found cyclic levels of progesterone indicative of ovarian activity as early as 20 days postpartum, concluding that Holstein cattle, at least under their conditions, were capable of cycling just as early as animals raised under more temperate conditions. These data indicate that given suitable management systems, specialised cattle can outperform more traditional, rustic stock. However, adequate conditions demand an investment in suitable housing facilities, which many producers in the tropics cannot meet.

2.3. Insufficient economical resources of the farmer

It has been said that reproduction is a luxury function and poorly fed animals will have a difficult time to reproduce adequately. As farmers in the developing world have serious problems in producing a constant food supply, it is not surprising to find poor nutritional status of cattle as a major setback to the reproductive efficiency of the herd. This situation is usually aggravated when farmers depend solely on products closely related to the rainy season and can offer little or no supplementation during the dry season. As this has been a usual problem in developing countries, studies in which scientists have tried to identify constraints in animal reproduction have always noted that year-to-year variation in the rainy season precludes the drawing of firm conclusions as to a policy to establish, for example, the choice of a breeding season or strategies for reproductive management in the dry and wet seasons, two distinct conditions. In the past, semi-intensive units were programmed to have a low economic investment with a modest income from selling meat or milk. Therefore, reproductive programmes were kept to a minimum, as there was not an immediate economic pressure to become more efficient. However, in many countries, demand for land from the less affluent sectors of society, has created the need for farmers to become more efficient in the use of the land. Therefore, it is not unusual to find excellent examples of adequate supplementation or even better, good management decisions in the use of improved pastures. As a consequence of these shortcomings, professionals involved in the sector of animal health and production have an important challenge in front of them, that of assisting farmers in planning strategies to maintain cattle in a good plain of nutrition based on the skilful use of the local resources.

TABLE II. AVERAGES OF REPRODUCTIVE PARAMETERS IN CATTLE RAISED FOR DAIRY, BEEF AND DUAL PURPOSE UNDER MEXICAN CONDITIONS (VALUES WITHIN PARENTHESIS INDICATE THE NUMBER OF STUDIES)

Reproductive Parameter (days)	Dairy	Beef	Dual Purpose
Age at puberty	457 [a]	529 [a]	547 [a]
	(10)	(13)	(15)
Age at first service	586 [a]	691 [a]	729 [a]
	(10)	(20)	(12)
Age at first conception	602 [a]	779 [b]	885 [c]
	(14)	(26)	(23)
Age at first calving	925 [a]	1049 [b]	1133 [c]
	(35)	(29)	(68)
Interval from calving to first oestrus	65 [a]	68 [a]	94 [b]
	(35)	(35)	(50)
Interval from calving to first service	71 [a]	85 [a]	129 [b]
	(17)	(16)	(25)
Interval from calving to conception	139 [a]	150 [a]	154 [a]
	(56)	(64)	(76)
Calving interval	435 [a]	437 [a]	426 [b]
	(73)	(78)	(112)

Uncommon letters within rows indicate a significant difference ($P < 0.05$)

2.4. Choice of breeding systems, artificial insemination (AI) versus natural mating

In relation to semen preparation, judged by the case reports obtained from the users of AI, the quality of the frozen semen prepared nationally, or imported from abroad, is quite satisfactory. Also, providers of the services related to storage and AI equipment have kept abreast of new developments, usually emanating from the industrialised world. Although not proven, a possible constraint to the industry has been the inadequate preparation of the technical staff inseminating cows. Still, in many developing countries, veterinarians in charge of the services on the farm, carry out the insemination procedure, whilst making their daily visit. Consequently, cows in the farm are inseminated at a predetermined time, irrespective of when they start showing signs of oestrus. Although these personnel have been professionally trained to provide veterinary services, one must not overlook the possibility that they are poorly trained in AI procedures; once again, research is needed to clarify these important points.

In farms where a layman carries out the AI technique, in many instances they have had little exposure to the use of AI prior to their training. Technical schools in charge of providing them with adequate practice are scarce. Furthermore, retraining, a most important consideration of AI technicians [14] is almost non-existent in developing countries. Surveys to evaluate the performance of AI technicians are mandatory if AI is to improve in the region.

An important consideration of decline in the use of AI is the high number of cows requiring repeat services. A common factor related to his failure is the increasing number of dual-purpose herds with clinical signs of uterine infections, particularly in the early postpartum period. Although there is a pressing need to elucidate the causes of this shortcoming, surprisingly there is little research authored by scientists in the developing world contributing to solve this problem. More important, whereas in one location this phenomenon is rather apparent, other similar areas have failed to report any incidence at all. Figures estimating repeated failure to conceive after AI have shown that at least 25% of the herd is not getting pregnant after three or more inseminations [15].

Finally, one cannot overlook the possibility that oestrus detection in these rather semi-intensified farming systems, is a major cause of concern. It has been calculated [9] that farmers are only detecting about 40% of the animals in oestrus.

3. IMPLEMENTATION OF FEEDING PRACTICES

Nutrition is considered to be one of the most important factors affecting cattle performance in the tropics, due to the low quality of tropical forages (low protein and energy) and seasonal variation in dry matter production. Thus, farmers in the lowland tropics are faced with the challenge of finding suitable sources of protein and energy with agricultural by-products available locally. Therefore, several supplementary feeding strategies have been proposed to surpass this shortcoming. Development of feeding and management systems based on fresh sugar can are feasible, whereby each hectare planted with sugar can may be converted to 15 000 kg of milk or 500 kg meat per year in dual purpose production systems in the tropics [16].

Strategic supplementation with bypass protein and fat for dual-purpose cattle has been assessed in the Colombian tropics during the dry season [17] and it was concluded that supplementing cows gave better total productive performance due to lower weight losses, higher milk production, better pregnancy rates an heavier calves at birth.

Another alternative of supplementation has been implemented with the use of molasses. Molasses-urea based supplements have been evaluated in dual purpose cows raised in the humid tropics of Mexico. For instance Castillo and co-workers [18] found that milk production was 0.8kg/cow/day higher in cows supplemented with molasses plus 3% urea. Another alternative of giving molasses is through multinutritional blocks based on urea molasses UMMB. The primary purpose of these supplements is to provide a broad spectrum of major and trace minerals [19]. Therefore by manipulating the rumen microflora through feeding UMMB an improvement in the digestive efficiency of crop residues can be achieved [20]. In Indonesia, Hendranto and co-workers [21] showed that Friesian dairy cows given UMMB supplementation increased total milk yield by 28% compared with unsupplemented controls over the 18 weeks of lactation. Mata and Combellas [22] found that inclusion of cottonseed (30%) in UMMB improved fertility and reduced liveweight losses. In addition supplementation through multinutrient blocks before the calving season affected the reproductive performance of cows with low body condition score [12].

In some countries legumes have been used as a source of protein. The tree legumes, e.g. *Gliricidia, Erythrina* and *Leucaena* have great potential as sources of legume fodder [19]. The high protein content of the leaves of *Gliricidia sepium* makes it particularly useful as an animal feed, especially as a complement to poor quality forages [23].

The overall performance of pasture-based production systems depends largely on the quantity of feed consumed and used beyond maintenance by the animal (6.kg of DM/100kg LW, and 7% of crude protein) [24]. Pasture management can affect productive performance in dual-purpose cows. The best balance between high production (milk production/ha and cow) and reproductive performance was found in the high stocking rate farm (1.5 cows/ha) in the humid tropics of Mexico

[25]. However this must be interpreted with caution as variations are to be expected in the general management of the farms and the forage availability throughout the year.

There is a pressing need to investigate on suitable methods of supplementation, particularly those economically feasible to farmers in dual-purpose systems. This principle becomes of primary importance as the twenty first century approaches for two reasons. First, traditional beef cattle enterprises based on extensive systems are facing serious problems for adequate implementation, for example countries like Costa Rica, with long tradition in raising beef cattle stocked 7.5 million heads in 1975. Nowadays, these numbers have been reduced to three million. Secondly, dual-purpose cattle systems are becoming more common in the tropics as it gives low-income farmers the economical diversity needed for subsistence.

4. ADEQUATE MARKETING OF DAIRY PRODUCTS IN THE TROPICS

Milk production is highly seasonal in the tropics, this is all concentrated in the rainy season and little milk is available in the dry season. This shortcoming creates two problems: a) collection of milk is circumscribed in certain periods of the year, leaving the investment, such as milking lorries and cooler containers, poorly used in certain periods of the year; b) infrastructure for preservation of milk, such as pasteurisation, long lasting skim milk, or even dehydrated as powder, is not economically feasible as again the plant will be working at capacity only part of the year. Alternatives such as breeding cattle to calve in the dry season are in need. However, feeding alternatives to complement grazing have to become feasible, as only milk produced exclusively from grazing is highly variable, since climatic changes can alter the growth of pasture by 50% in a period of only 7 to 10 days. Also, Aluja and McDowell [26] found that average production above 8 litres required supplementation. Otherwise reproductive performance could be affected.

More research is obviously needed in all the headings indicated in this review, particularly how we could increase our crossbreeding programmes of *Bos taurus* x *Bos indicus* breeds in accord with improving feeding practices.

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USE OF A STANDARDIZED PROTOCOL TO IDENTIFY FACTORS AFFECTING THE EFFICIENCY OF ARTIFICIAL INSEMINATION SERVICES FOR CATTLE THROUGH PROGESTERONE MEASUREMENT IN FOURTEEN COUNTRIES

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Abstract

USE OF A STANDARDIZED PROTOCOL TO IDENTIFY FACTORS AFFECTING THE EFFICIENCY OF ARTIFICIAL INSEMINATION SERVICES FOR CATTLE THROUGH PROGESTERONE MEASUREMENT IN FOURTEEN COUNTRIES

The aim of this co-ordinated research project (CRP) was to quantify the main factors limiting the efficiency of artificial insemination (AI) services in cattle under the prevailing conditions of developing countries, in order to recommend suitable strategies for improving conception rates (CR) and the level of usage of AI by cattle farmers. A standardized approach was used in 14 countries over a five-year period (1995–1999). The countries were: Bangladesh, China, Indonesia, Myanmar, Pakistan, Sri Lanka and Vietnam in Asia; and Argentina, Chile, Costa Rica, Cuba, Peru, Uruguay and Venezuela in Latin America. A minimum of 500 cows undergoing first insemination after calving were expected to be monitored in each country. Data regarding farms, AI technicians, semen used, cow inseminated, characteristics of the heat expression and factors related to the insemination were recorded. Three milk samples (or blood samples for dairy heifers and beef cows) were collected for each service to measure progesterone by radioimmunoassay. These were collected on the day of service (day 0) and on days 10–12 and 22–24 after service. Field and laboratory data were recorded in the computer package AIDA (Artificial Insemination Database Application) which was developed for this CRP. The study established the current status of AI services at selected locations in participating countries and showed important differences between Asian and Latin American farming systems. The mean (± s.d.) of the interval from calving to first service for 7 992 observations was 120.0 ± 82.1 days (median = 95 days) with large differences between countries ($P < 0.05$). The overall CR to first service was 40.9% (n = 8 196), the most efficient AI services being the ones in Vietnam (62.1%), Chile (61.9%) and Myanmar (58.9%). The interval between the first and second service was 44.6 ± 44.4 days (n = 1 959). Progesterone data in combination with clinical findings showed that 17.3% of the services were performed in non-cyclic cows; within this group 10.4% of cows were anoestrous or anovulatory, 2.2% were non-pregnant but bearing an active corpus luteum, and 4.7% were already pregnant. The highest incidence of problems was observed in farms of Cuba, Costa Rica, Indonesia and Venezuela. Progesterone data also showed that 27.4% of inseminated cows did not conceive but subsequent heats were unobserved, resulting in their non-pregnancy status being identified only at manual pregnancy diagnosis, usually 80–120 days after service. A further 10.1% of inseminated cows most probably conceived but lost their embryos between 16 and 50–60 days after service. These results show that nearly half of the inseminations were associated with factors of management deficiency or human error in the farms or in the AI service, thus adversely affecting the reproductive performance of the herds and leading to low efficiency of AI services.

1. INTRODUCTION

Specialized dairy farms and some dual-purpose (milk and beef) farms routinely use artificial insemination (AI) for breeding and genetic improvement; however, a number of biological, managerial and socio-economic factors affect the quality and efficiency of the technique and therefore limit the number of farmers embracing its use. On the other hand, actions aiming to improve the efficiency of the AI system have to be based on proper knowledge of the prevailing conditions and constraints.

Systematic and detailed collection of information regarding the farming system, farm characteristics, animal performance, application of the AI technique and heat expression may enable the identification of the limiting factors affecting the reproductive performance of dairy herds. Moreover, the measurement of progesterone using radioimmunoassay (RIA) in a series of milk samples (or blood in the case of dairy heifers and beef cattle) can add valuable information to evaluate the reproductive status at and after AI, and to determine factors that affect its efficiency. It is also a proven tool to monitor the responses to technical interventions aimed at ameliorating constraints to AI [1, 2, 3]. The results of these interventions can be reflected in improved efficiency in heat detection, better timing of AI and higher CR. These are key factors to increase the number of animals and farms being bred by AI. An additional benefit would be the identification of animals with pathological conditions such as anoestrus beyond 60 days after calving).

This paper presents a synthesis of the methodology adopted, and the results obtained, under the Joint FAO/IAEA Co-ordinated Research Project (CRP) entitled "Use of RIA and Related Techniques to Identify Ways of Improving Artificial Insemination Programmes for Cattle Reared under Tropical and Sub-tropical Conditions", which was implemented in 14 developing countries over a period of 4 years. The objective of this paper is to compile salient information from individual country projects, in order to highlight the potential value of the methodology used (i.e. progesterone RIA, clinical data, field data-entry forms and a computer database) for improving the efficiency of AI systems in developing countries. It is emphasized that the aim is not to compare the efficiency of AI services or to highlight differences between countries, and that the results do not apply at national level, but reflect the status of AI services at specific locations selected for the study in the different countries. The authors fully acknowledge the scientific rights of the project counterparts to the data obtained under this project, and wish to refer the reader to the individual papers published by them in this IAEA-TECDOC for the detailed results.

2. MATERIAL AND METHODS

2.1. Selection and technical backstopping of the participating institutions

The full description of the project including the rationale, scientific scope, operating procedure and requirements for participation were published in an IAEA Newsletter in 1994 [4] and distributed worldwide through various means including FAO and UNDP offices in developing countries. Around 30 proposals for research contracts were received, mainly from institutes in Asian and Latin American countries. Selection of suitable proposals was done on the basis of technical capabilities, adequate links with large AI services and availability of facilities and expertise on progesterone RIA. The institutions selected were from Bangladesh (BGD), China (CPR), Indonesia (INS), Myanmar (MYA), Pakistan (PAK), Sri Lanka (SRL), and Vietnam (VIE) in Asia (n = 7), and from Argentina (ARG), Chile (CHI), Costa Rica (COS), Cuba (CUB), Peru (PER), Uruguay (URU), and Venezuela (VEN) in Latin America (n = 7).

The chief scientific investigator (CSI) of each research contract participated in a research coordination meeting (RCM) in November 1995 in Vienna, to standardize work plans and key activities between participating countries, and to be trained on the use of AIDA (Artificial Insemination Database Application) a computer software package developed for this project [5]. A second RCM took place in February 1997 in Melbourne, Australia to review preliminary results and define technical interventions. The third and final RCM took place in May 1999 in Uppsala, Sweden to present the final results and prepare papers for publication. A group of advisers in the field of animal reproduction, artificial insemination, semen evaluation, and RIA technically supported the contract holders during the meetings and through correspondence at other times.

In addition, some of the authors visited many of the project sites during the implementation of the survey. Copies of the data tables recorded on AIDA were also interchanged at various times by e-mail for review and advise.

2.2. Data collection

Field activities were conducted mainly during 1996 and 1997 in the 14 participating countries, but in a few places this period was extended until 1998. The laboratory work was completed by May 1999.

Information regarding the farms involved in the survey, AI technicians, semen and sires, inseminated animals, heat expression observed by farmers and AI technicians, and characteristics of the local AI services was collected. Five data-entry forms were prepared to collect field information (i.e. farm, AI technician, semen batch, cow, insemination). The forms, which were translated into the local languages, were designed in a way that most of the information could be entered by ticking off the options in a list, and little information had to be written down. All paper forms resembled the on-screen forms of the computer database to facilitate data entry. The list of recorded parameters, data recording forms, and further explanation can be found in García, 1996 [5].

It was expected that the survey would cover a minimum of 500 cows undergoing the first insemination after parturition. However ARG, CUB, MYA and PAK could not reach this target. The farms were selected on the basis of their being representative of the farming system in the study area. Elite farms or those with higher than average productivity or reproductive performance were avoided. AI technicians were not informed of the real objectives of the study to avoid any bias in the results due to changes in their attitude and performance.

2.3. Milk (and blood) sampling and laboratory analysis

Three samples were collected. The first was taken on the day of service (day 0), the second between days 10–12 after service, and the third between days 22–24 after service. The third sample was only collected if the animal was not observed in heat at the expected time. The sampling scheme was repeated for subsequent services in some countries.

A typical milk sample consisted of 5–20 mL of milk collected from any healthy quarter of the mammary gland into vials with sodium azide as a preservative, kept in refrigeration (+4°C) for 3–7 days and then defatted by centrifugation (2 000 g ×15 min). Blood samples (5–10 mL) were usually collected from the jugular vein into heparinized vacutainers, centrifuged within 4 hours, and the plasma harvested. Skimmed milk and plasma samples were kept at –20°C until analysis.

Progesterone concentration in milk or plasma samples was measured using the FAO/IAEA RIA kits [6] which are based on a solid-phase RIA technique employing ^{125}I-progesterone as tracer.

2.4. Data storage and statistical analysis

An *ad hoc* database using Microsoft Access 2.0 and runtime files was developed to enter the field data and laboratory results [5]. This software entitled "AIDA" (Artificial Insemination Database Application) had on-screen data-entry forms for farms, AI technicians, semen batches, inseminated cows, services, and progesterone values. The application contained facilities for data verification and a large number of built-in reports with simple statistical analysis for calculating reproductive performance and the interpretation of progesterone values. AIDA also had data export routines for a number of datasets to export raw and calculated data in Excel 3.0 format.

The statistical analyses used in the study are described in "GAIDA" (Guide for AIDA Data Analysis) [7].

3. RESULTS AND DISCUSSION

3.1. Characteristics of farms and AI services

3.1.1. Farms

The surveys in Asian countries, except in CPR, focused mostly on smallholder farms (1-2-cows) but some 10–20 cow-farms were also included. Data from INS and SRL included two distinct types of farms (smallholdings and large farms) and the data was therefore analysed separately for each type of farm. As shown in Table I the predominant type of animals were dairy cattle, with some dual-purpose

TABLE I. PREDOMINANT CHARACTERISTICS OF FARMS AND FACTORS RELATED TO INSEMINATIONS IN THE 14 COUNTRIES PARTICIPATING IN THE PROJECT

Country	N° of Farms	Purpose	Housing	N° of cows/farm	Milkings per day	Suckling	Km from AI unit to farm	Heat detection	Time of AI (% am)	AI - PD[4] interval (d)
Argentina	8	Dairy	None	180	2	None	0	Visual	40.5	?
Chile	7	Dairy[1]	Night corral	150	2	None	13.1	Visual	85.4	109.8
Costa Rica	4	Dual	Corral	74	1-2	Several	0	Visual	23.4	67.1
Cuba	4	Dairy	Corral	286	2	Several	0	Visual	100.0	?
Peru	9	Dairy	Corral	350	2-3	None	0	Visual	49.3	?
Uruguay	5	Dairy	None	180	2	None	3.4	Visual	97.6	?
Venezuela	6	Dairy[1]	None	476	2	2 x day	19.7	Visual[3]	59.2	62.6
Bangladesh	406	Dairy[2]	Tie stall	2	1-2	Several	2.6	Visual	41.7	81.6
China	10	Dairy	Corral	266	2	None	0	Visual	?	60.4
Indonesia (a)	229	Beef[2]	Various	2	--	--	8.2	Visual	62.1	112.8
Indonesia (b)	1	Beef	Corral	7 500	--	--	40.0	Visual	78.4	83.8
Myanmar	222	Dairy	Tie stall	2	1-2	Several	3.5	Visual	41.4	83.4
Pakistan	107	Dairy	Tie stall	2	2	2 x day	10.0	Visual	100.0	?
Sri Lanka (a)	246	Dairy	Tie stall	1	2	2 x day	5.2	Visual	52.9	81.0
Sri Lanka (c)	4	Dairy	Tie stall	230	2	None	0	Teaser	4.3	67.0
Vietnam	467	Dairy	Tie stall	3	2	2 x day	4.6	Visual	59.3	100.2

[(a)]Small farms; [(b)] Co-operative farm; [(c)] Government farm

[1] Some cows for dual purpose; [2] Some cows for draught; [3] Some farms use teaser bulls; [4] Interval from AI to pregnancy diagnosis (mean in days)

animals and, in the case of INS, mainly beef cattle. Hand milking was done 1–2 times per day using the calf for the "let-down" of milk. Farm records were non-existent or limited to cow cards.

The Latin American studies all had large farms with cattle populations ranging from 20 to 1 500 dairy cows, mainly of the Holstein type. Cattle were kept in corrals (CHI, COS, CUB, PER) or grazed in large paddocks (ARG, URU, VEN). Milking was done twice a day by machine and without the presence of the calf (Table I). Farm records were mainly kept in computers and in printed cow cards.

3.1.2. Artificial insemination units

Farmers contacted the AI units for insemination services using various mechanisms. Inseminators had to travel long distances to reach Latin American farms that did not have their own inseminators (CHI, VEN), while under Asian conditions, most of the farms were located within 1–7 km from the AI unit (Table I) and inseminators used bicycles, motorcycles or cars for transportation.

The time for providing AI services in relation to the time when heat was first observed did not have a common pattern. All services in PAK and CUB and most of the services in CHI and URU were performed in the mornings (Table I), whereas other places had an even distribution between morning and afternoon services. The interval between heat observation and AI was only recorded in 7 countries and some intervals were unusually long (BGD 17.6 h, SRL 20.9 h and VIE 22.4 h). Pregnancy diagnosis by rectal palpation was usually done between 80–120 after the service (Table I).

3.1.3. AI technicians

The number of inseminators included in the survey varied between 2 and 19 (mean: 8.3 and median: 7) per country. The inseminators in Asia were mainly employed by government AI centres whereas in Latin America they were regular staff of private farms and co-operatives. Large variability was found among the average number of services done by a technician per month and in their educational background (Table II).

3.1.4. Semen and sires

The semen used was produced locally in most countries, except in PER and COS which mostly used imported semen. Frozen straws were the most common type of semen; however pellets were also used, either partly (CPR and VIE) or exclusively (CUB). Some chilled semen was used in BGD and SRL. An interesting difference was that Asian AI services used 0.25 mL straws whereas the Latin Americans used 0.5 mL straws (Table II). Thawing of frozen semen was done mainly in warm water.

The number of sires involved in the study varied from 7 to 68 per country (mean 29.9, median 25, Table II). The quality of the semen, when it was evaluated, was usually within acceptable standards, with the exception of PAK where motility and viability of sperms were low at the AI units due to inappropriate storage and handling.

3.1.5. Inseminated cows

As shown in Table III the age at first service after parturition in the surveyed population was 5.6 ± 2.3 years (mean ± s.d, n = 5 476) and parity was 2.8 ± 1.7 (n = 5 953), without significant differences between countries. This homogeneous nature of the population as age and parity was concerned, facilitated the possibility of comparing other variables in the study.

The need for some sort of professional assistance during parturition was high in ARG (16.7%), CHI (30.2%) and URU (11.5%). It is a common practice in these large farms to keep cows close to parturition time in delivery corrals and around-the-clock specialized staff observing the animals and intervening if calving is taking longer than 1–2 h. A high incidence of retention of placenta was observed in VIE (9.9%).

Body condition and milk yields at AI were abnormally low in Holstein cows in CUB (1.9 and 4.8 kg, respectively) due to lack of concentrates and scarcity of forage in the country. Body weight at AI greatly differed between countries, mainly due to the variety of breeds and nutritional regimes. The higher body weights were observed in PER (591 kg), CHI (546 kg) and URU (536 kg), and the lowest in BGD (211 kg), the latter reflecting the small size of the indigenous animals (Table III).

TABLE II. PREDOMINANT CHARACTERISTICS OF SEMEN USED AND AI TECHNICIANS IN AN AI SURVEY CONDUCTED IN 14 COUNTRIES.

Country	N° of sires	Semen			AI Technician				
		N° of batches	Source	Semen type (volume, ml)	N°	Age (years)	Level of education	AI per Month[1]	Employer
Argentina	30	32	Local[2]	Straw (0.25)	10	40.0	High /Primary school	23	Private /government farm
Chile	52	65	Local[2]	Straw (0.5 /0.25)	6	48.5	Diploma/Primary	195	Self employed / various
Costa Rica	11	15	Imported	Straw - ?	4	28.2	Primary school	?	Private farm
Cuba	19	20	Local	Pellets (0.5)	7	33.3	Diploma	150	Government farm
Peru	68	68	Imported	Straw (0.5)	19	?[5]	Various	20	Private farm
Uruguay	24	24	Local[2]	Straw (0.5)	3	39.6	Primary / High school	135	Private /government farm
Venezuela	51	60	Local	Straw (0.5)	10	36.3	High /Primary school	90	Private farm /co-operative
Bangladesh	26	145	Local[2]	Straw /Chilled[3]	7	39.9	Diploma	165	Government AI centre
China	67	67	Local	Straw /Pellets (0.25)	8	34.2	High / Professional	?	?
Indonesia (a)	6	14	Local	Straw (0.25)	14	29.1	High /Primary school	?	Government AI centre
Indonesia (b)	2	5	Local	Straw (0.25)	2	?	High school	?	Government AI centre
Myanmar	11	45	Local	Straw (0.25)	5	48.4	Diploma	65	Government AI centre
Pakistan	7	7	Local	Straw (0.5)	2	28.8	Diploma	35	Government AI centre
Sri Lanka (a)	24	68	Local[2]	Straw /Chilled[4]	8	42.9	High school	54	Government AI centre / farm
Sri Lanka (c)	7	7	Imported	Straw (0.25)	7	34.2	High school	43	Government farm
Vietnam	13	13	Local[2]	Pellets /Straw (0.5)	4	35.5	Professional / Diploma	200	Government AI centre

[a] Small farms; [b] Co-operative farm; [c] Government farms
[1] Average N° of inseminations per month; [2] Some were imported; [3] Various volumes; [4] 0.25/1.0; [5] Unknown

TABLE III. PREDOMINANT CHARACTERISTICS OF INSEMINATED ANIMALS AND HEAT EXPRESSION IN AN AI SURVEY CONDUCTED IN 14 COUNTRIES

Country	N° of cows	Main breed	Parity	BCS at AI	BW at AI (kg)	Milk yield at AI (kg)	Heat signs	Swelling of vulva	Pipette passage (%)[1]
Argentina	163	Holstein	2.6	2.7	?	24.4	Standing	Marked[2]	8
Chile	713	Friesian	2.6	2.8	546	21.8	Standing	Marked[2]	18
Costa Rica	484	Crossbred	3.7	?	?	?	Standing /Mounting	?	8
Cuba	251	Holstein	3.4	1.9	394	4.8	Standing	Marked	5
Peru	732	Holstein	2.7	2.7	591	24.7	Standing	Various	1
Uruguay	728	Holstein	2.5	2.2	536	19.4	Standing	?	0
Venezuela	499	Crossbred	3.2	3.0	445	7.9	Standing	Various	0
Bangladesh	444	Indigenous	2.5	2.9	211	2.7	Mounting / Bellowing	Marked[2]	12
China	2 018	Holstein	?	?	?	?	?	?	?
Indonesia (a)	359	Bali	3.0	3.6	287	- -	Mucous /Standing	Marked	0
Indonesia (b)	248	Brahman	2.5	2.9	320	- -	Various	Marked	16
Myanmar	270	Crossbred	3.4	3.3	378	10.4	Mucous /Mounting	Marked	3
Pakistan	110	Various	3.0	3.5	358	5.5	Mucous /Mounting	Slight	0
Sri Lanka (a)	265	Various	2.7	2.6	296	6.3	Mucous /Bellowing	Marked[2]	2
Sri Lanka (c)	126	Various	3.1	2.8	408	11.0	Various	Marked[2]	2
Vietnam	583	Holstein	2.2	?	413	16.0	Mucous/Standing	Marked	12
Total / Mean:	7 993		2.8	2.8	422	13.3			

[(a)] Small farms; [(b)] Co-operative farm; [(c)] Government farms
[1] Frequency of difficulty in passing the pipette through the cervix; [2] Slight in some cases

3.1.6.Heat expression and AI

The presence of mucous discharge in the vulva area was the most frequently used sign of heat expression under tie-stall Asian farm conditions. Other signs such as the cow mounting others or the cow allowing to be mounted were important in farms with several animals (Table III). On the other hand, in Latin American farms the standing cow was the basic sign for AI and other heat signs were rarely accepted. Heat synchronization was applied to inseminate some cows in INS and for most of the animals in CUB.

Inseminators generally reported a marked swelling of the vulva at the time of AI, otherwise a slight swelling. The level of difficulty in passing the AI pipette through the cervix (easy, difficult, impossible) was reported as difficult in ≥10% of the services by inseminators in BGD, CHI, IND (large farm only) and VIE (Table III).

3.2. Reproductive performance

Table IV shows the reproductive performance of inseminated cows in the 14 areas of the surveys. The difference in the number of observations between the interval to first service (n = 7 991) and conception rate to first service (n = 8 196) was due to missing calving dates and unavailability of inseminated cows for the pregnancy diagnosis test in several countries. CPR had the largest dataset (2 018 cows) and PAK had the smallest (110 cows).

The overall calving to first service interval was 121.5 ± 82.1 days (median = 95 days) with large differences between countries. The shortest intervals were in PER, CHI and ARG, probably due to the higher genetic quality of the animals, better nutrition and management, and more suitable climatic condition for rearing Holstein-type cattle. The long interval observed in CUB can be attributed to poor nutrition due to the severe national economic constraints during the survey period. This interval was longer in most Asian countries, especially in indigenous cattle of BGD and PAK (around 200 days) and in small beef farms in INS (around 270 days).

The overall mean for the calving to conception interval was only 16 days longer than the interval to first service. This figure has to be interpreted with caution as it represents less than 50% of the inseminated population. Many cows did not get pregnant and were culled, while many others may have got pregnant at a later stage but to services that were not monitored.

The overall conception rate to first service (CRFS) was 40.9%. Conception rate to all services was not calculated for the purpose of this summary, since a considerable proportion of cows that returned in heat were not monitored further. The highest CRFS were obtained by AI services in VIE (62.1%), CHI (61.9%) and MYA (58.9%) whereas those from CUB, INS, PAK, and PER had the lowest rates. The reasons identified for poor performance in CUB were those already described plus the fact that many cows with low body condition were subjected to heat synchronization. In PAK it was due to poor handling of frozen semen at the AI units, while in PER cattle suffered the effects of unusual heat stress due to "El Niño" which may have caused early embryonic death and subsequent anoestrus [8]. A high proportion of beef cattle in INS (in both types of farms) were inseminated during the luteal phase, as will be shown later.

Differences in CRFS due to farm size, body condition at AI, ease of pipette passage, years of experience of the AI technician, swelling of vulva at AI and semen source were evaluated in countries having a balanced number of animals between groups. Farms in VIE and MYA with 1–2 cows or with 5–20 cows had similar CRFS, whereas in COS and VEN large farms (>300 cows) had lower rates (14.8 and 22.2% respectively) compared with medium size farms (20–300 cows, 44.4 and 53.3%, respectively) ($P < 0.05$). This difference was not significant in medium (47%) versus large farms (56.0%) in CHI. Body condition at AI proved to be important when datasets from AI systems in BGD, CHI, SRL and VEN were pooled (Figure 1, n = 1 494). Cows with a score of 1.0–2.4 (on a 1–5 scale) had lower CRFS (33.1%, $P < 0.05$) than cows with BCS of 2.5-3.5 (40.0%) and >3.5 (40.4%).

Pooled data from BGD, SRL, VIE, ARG, CHI, COS and CUB (n = 2 330) showed differences in CRFS ($P < 0.05$) between inseminations carried out by inseminators with < 7 years of experience (24.9%) and those with 7.1–12.0 years (44.2%) and > 12 years (43.5%). The degree of swelling of the vulva at AI (none, slight and marked) did not show any significant relation with CRFS (n = 1 519, $P > 0.05$), and local semen had similar performance to imported semen in SRL and URU, but showed lower CRFS in BGD (38.9 vs. 57.5%, $P < 0.05$).

TABLE IV. INTERVALS (DAYS) FROM CALVING TO FIRST SERVICE AND TO CONCEPTION, AND CONCEPTION RATE TO FIRST SERVICE IN AN AI SURVEY CONDUCTED IN 14 COUNTRIES

	Calving to 1st Service Interval (d)				Calving to Conception Interval (d)				Conception Rate[1]	
	n.	Mean	s.d.	Median	N	Mean	s.d.	Median	n	%
Latin America	3 583	99.8	69.6	80.0	1 981	122.3	76.0	96.0	3 773	38.9
Argentina	365	91.1	56.5	80.0	149	92.3	47.7	81.0	366	41.6
Chile	663	88.7	56.9	74.5	490	107.9	73.3	83.5	713	61.9
Costa Rica	497	118.1	85.0	91.0	270	141.6	99.7	104.5	484	39.7
Cuba	250	158.4	109.6	112.0	38	174.9	108.4	144.0	251	15.1
Peru	617	80.5	35.2	71.0	249	113.5	61.3	93.0	732	25.7
Uruguay	692	101.5	48.8	88.0	601	133.9	61.0	124.0	728	40.4
Venezuela	499	95.0	77.4	68.0	184	120.1	89.5	93.5	499	32.5
Asia	4 408	137.3	87.3	111.0	2 746	146.7	84.7	124.0	4 423	42.6
Bangladesh	449	195.9	86.9	184.0	232	195.9	88.4	184	444	46.2
China	1 951	113.7	74.8	91.0	969	138.4	92.2	112.0	2 018	37.8
Indonesia (a)	332	150.4	89.9	119.0	191	147.4	72.6	124.0	359	27.6
Indonesia (b)	243	269.5	97.0	227.0	65	255.1	91.3	223.0	248	24.6
Myanmar	361	104.6	41.4	94.0	264	110.4	42.9	97.0	270	58.9
Pakistan	87	205.6	131.7	166.0	30	209.1	119.2	166.0	110	27.3
Sri Lanka (a)	272	174.4	87.9	155.0	256	190.8	91.5	177.5	265	53.6
Sri Lanka (c)	133	111.2	74.2	87.0	171	156.5	92.7	136.0	126	49.2
Vietnam	580	107.5	34.6	104.0	568	118.9	38.7	115.0	583	62.1
Total:	7 991	121.5	82.1	95.0	4 727	137.6	81.3	115.0	8 196	40.9

[a] Small farms; [b] Co-operative farm; [c] Government farms
[1] Conception rate at first service

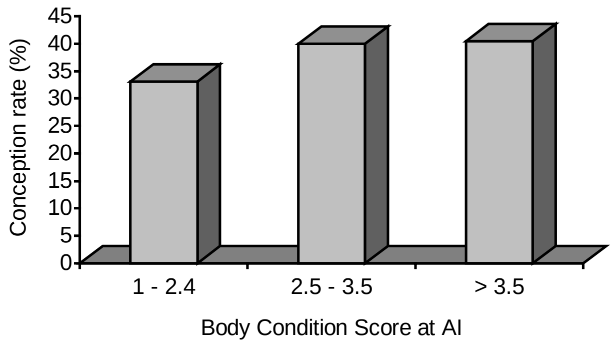

FIG.1. Effect of body condition score (1–5 scale) at artificial insemination (AI) on conception rate to first service in dairy and dual-purpose cows from Bangladesh, Chile, Sri Lanka and Venezuela.

The inter-service interval between the first and the second service in 1 959 observations was 44.6 ± 44.4 days. This is equivalent an average of one missing heat between services. Serious problems in heat detection were observed in the COS and CPR AI services as the intervals were >57 days.

3.3. Problems identified in the AI services

Basically, the sample on the day of service was used to verify whether the animal was inseminated during the luteal phase (i.e. incorrect timing, high progesterone values); the two-sample set (day of service and at mid-cycle) was used to verify whether the animal was cycling and had ovulated; and the three-sample plus pregnancy diagnosis strategy (the third sample collected at day 22–24 after AI) was used to verify whether the animal had conceived, had lost the embryo, had been inseminated while already pregnant or had been inseminated when acyclic. The most common occurrences of progesterone values and the respective interpretation are shown in Table V.

Two major deficiencies were identified in the AI services through progesterone concentration from samples collected in more than 8 500 inseminations (Table VI). AI at inappropriate times occurred in 17.3% of all services denoting serious heat detection errors at farm level. These services were performed in non-cycling cows (10.4%) and in cows with active Corpora Lutea (CLs) at AI (6.9%); most of the latter were already pregnant. Inseminations in non-cycling cows was most common in CUB, INS (both farm types), COS and VEN (Table VII). Services in cows bearing an active CL occurred mainly in INS-large farm (17.8%), SRL-small farms (13.2%) and URU (11.8%).

The second major deficiency was related to post-AI estrous detection and herd management, which affected 37.5% of the inseminations (Table VI). In this case, 10.1% of the inseminated animals suffered late embryonic deaths (between 16–60 days after service) but were not reported in heat until pregnancy diagnosis took place, usually 80–120 days after service. Another group representing 27.4% of the inseminated animals failed to conceive to that service but subsequent heats remained undetected until pregnancy diagnosis was performed. Farms in CPR and URU had the highest late embryonic deaths (17%), whereas farms in CUB, PER and INS had the largest frequencies of cows with unobserved heats after failed inseminations (68.9, 45.3, and approx. 40%, respectively).

3.4. Practicability and constraints of the 3-sample strategy

The 3-sample set (milk or blood) collected at days 0, 10–12, and 22–24 in relation to the insemination, together with pregnancy diagnosis by rectal palpation, proved to be a sound strategy for evaluating the efficiency of reproductive management at farm level and the outcome of AI services. However, it is also important to point out that the system requires a large number of cows in the evaluation scheme in order to get a meaningful set of data. The number of animals will depend, among others factors, on the farming system, the genetic variability of the population, the number of variables to be considered in the analysis, and the efficiency of the sample collection.

TABLE V. INTERPRETATION OF RIA PROGESTERONE DATA ALONE OR IN COMBINATION WITH CLINICAL INFORMATION FOR THE EVALUATION OF AI SERVICES (HIGH: ≥3 NMOL/L; LOW ≤1 NMOL/L) USING MILK (OR PLASMA) SAMPLES COLLECTED AT DIFFERENT INTERVALS AFTER AI

Nº of samples	Day of collection			Pregnancy diagnosis	Interpretation
	0	10-12	22-24		
Three	Low	High	High	Positive	Pregnancy
	Low	High	Low	Negative	Non-fertilization, early embryonic mortality, (post AI-anoestrus)
	Low	High	High	Negative	Late embryonic mortality (>day 16), luteal cyst, persistent CL
	High	High	High	Positive	AI on pregnant animal
Two	Low	High	--	--	Ovulatory cycle
	Low	Low	--	--	Anoestrus, anovulation, short luteal phase
	High	High	--	--	AI on pregnant animal, luteal cyst
	High	Low	--	--	AI during the luteal phase
One	Low	--	--	--	AI at a time other than the luteal phase
	High	--	--	--	AI during the luteal phase

TABLE VI. FACTORS DETRIMENTAL TO THE AI SERVICE THAT WERE IDENTIFIED THROUGH THE 3-SAMPLE STRATEGY AND SUBSEQUENT PREGNANCY DIAGNOSIS

Main problem	Specific deficiency in the AI system	Population affected (%)
• Inappropriate AI probably due to wrong estrous detection		17.3
	o AI in cows with active *corpora lutea*	6.9
	o AI in non-cyclic cows	10.4
• Deficient estrous detection and herd management		37.5
	o Cows fail to conceive, subsequent heats are unobserved and found not-pregnant at pregnancy diagnosis test	27.4
	o Cows conceive but had late embryonic losses and are found not-pregnant at pregnancy diagnosis test	10.1

Collection of the first milk sample is not a major problem as it can be taken by the inseminator. The other two samples require someone to visit the farms or have to be done by the farmers (small farms) or farm employees (large farms) on the exact days and from the correct animals. In the case of the latter approach, some mechanism has to be in place for delivering the samples to the laboratory. A second constraint is that in 18.4% of the 3-sample sets of this study (n = 6 483) at least one sample showed intermediate progesterone values (>1 - < 3 nmol/L), and therefore the dataset become invalid for proper interpretation. Good quality RIA laboratories can reduce this figure through accurate assay results, but other factors such as mishandling of samples can contribute to this problem.

4. CONCLUSIONS

The standardized methodology used across countries and the strategy of combining data from the field and laboratory, together with uniform data-entry forms and a computer database, allowed the generation of unique information regarding reproductive management and AI systems in developing countries. It resulted in linkages between research institutes and AI centres and permitted identification of the main limiting factors at each specific location within the 14 Asian and Latin American countries participating in the project.

The survey showed that nearly half of the services were linked to a detectable and measurable deficiency, mainly related to a human error, which severely affected reproductive performance of cattle herds and also the quality and efficiency of AI services. Future efforts must be focused on alleviating the problems identified and will involve the following, depending on location: education of farmers on heat detection, herd management practices and better nutrition; improving the knowledge and skills of inseminators in AI practices; further research on embryo mortality, suckling practices and methods for heat induction/synchronization; improved semen handling, storage and quality control; and improved recording, evaluation of results and follow-up services to farmers by the providers of AI services.

Similar surveys would be valuable in other developing countries to improve the quality and efficiency of AI services.

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USING GAIDA (GUIDE TO AI DATA ANALYSIS) TO ANALYZE DATA COLLECTED FROM ARTIFICIAL INSEMINATION PROGRAMMES FOR CATTLE IN DEVELOPING COUNTRIES

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Abstract

USING GAIDA (GUIDE TO AI DATA ANALYSIS) TO ANALYZE DATA COLLECTED FROM ARTIFICIAL INSEMINATION PROGRAMMES FOR CATTLE IN DEVELOPING COUNTRIES.

The objectives of AIDA (Artificial Insemination Database Application) and its companion GAIDA (Guide to AI Data Analysis) are to address two major problems in on-farm research on livestock production. The first is the quality of the data collected and the second is the intellectual rigor of the analyses and their associated results when statistically testing causal hypotheses. The solution is to develop a data management system such as AIDA and an analysis system such as GAIDA to estimate parameters that explain biological mechanisms for on-farm application. The system uses epidemiological study designs in the uncontrolled research environment of the farm, uses a database manager (Microsoft Access) to handle data management issues encountered in preparing data for analysis, and then uses a statistical program (SYSTAT) to do preliminary analyses. These analyses enable the researcher to have better understanding of the biological mechanisms involved in the data contained within the AIDA database. Using GAIDA as a guide, this preliminary analysis helps to determine the strategy for further in-depth analyses.

1. INTRODUCTION

What is GAIDA? It is a “Guide to AI Data Analysis”, comprising a menu of statistical procedures using SYSTAT 6.01 [1], a statistical software package that is easy to use, is compatible with Microsoft Access through Microsoft Excel, and has good graphics capabilities. While GAIDA was developed specifically for AIDA (Artificial Insemination Database Application) [2], it can be adapted for use by other scientists to help analyze on-farm animal production or reproduction data.

GAIDA analyses include Stem and Leaf plots, Histograms, Descriptive Statistics, Scatter Plots, Correlation Matrix, Multivariate Regressions with Residuals, AVOVA and Least Square Means, and Frequency Tables. The initial analyses are designed to allow the researcher to become more familiar with the data, while later analyses are used to test relationships between variables in the data.

2. LOOKING AT DATA

2.1. Stem and leaf plot

First the researcher needs to examine his or her data. Constructing a stem and leaf plot allows one to check for any outlying data points and assess the normality of the distribution of the sampled data on one screen. The entire set of data is represented in one picture and provides the researcher with an opportunity to quickly identify any outlying data points that may represent errors in data entry or specific values that may need to be excluded in later analysis. Stem and leaf plots look like a histogram on its side, but provide more information. Constructing one in SYSTAT will provide you with the sample median, the hinges (25th and 75th percentiles), and the extremes, as well as the actual values in the leaves of the plot. Extremely large or small values are flagged as “outside values”. An example is given in Figure 1 from the data collected in Uruguay:

```
Stem and Leaf Plot of variable:  INTERV_AI, N = 739
     Minimum:    18.000
     Lower hinge:    69.000
     Median:    89.000
     Upper hinge:   123.000
     Maximum:   365.000

        1  8
        2  89
        3  46689
        4  11112233334555556666666777888999999
        5  000011111222222233333344444444455555566666666777777778888889999
        6 H 00001111112222222233333333344444455555555555666666666666667777777*
        7  0000000000111111111222222222222222222222223333333333333334444444*
        8 M 00000000001111112222222223333333333444444444444444445555666666*
        9  00000001111111122222222333333333344444445555555556666667777777788888*
       10  00000111111222233334444444455555556666666777777777778888999999
       11  000000222233333344444455556677777788889 9
       12 H 00001112233333444444555666666667888
       13  0001111222223333333444555567899
       14  0011112233344444455667788899
       15  00022233444444455567899
       16  00112223445568
       17  245789
       18  112233466699
       19  11234577789
       20  113344
   * * * Outside Values * * *
       20  567889
       21  3477
       22  0067
       23  057
       24  022368
       25  0235559
       27  4
       28  29
       30  78
       31  2
       35  3
       36  5
```

FIG.1. Stem and leaf plot of interval to AI

Looking at the Stem and Leaf Plot, it is noted that the distribution of interval from calving to AI (INTERV_AI) is skewed to the left. Additionally about 40 values are greater than 2 standard deviations from the mean. Thus 5% of the days to first breeding are much longer than the normal population.

2.2. Histograms

Another method to graphically look at the data is to make a Histogram. A Histogram shows the number of observations in each category. It also gives one an idea of the normality of the data’s distribution. The data graphed in Figure 2 is the same as in the stem and leaf plot.

One will note the distribution of INTERV_AI is skewed to the left and that most of the observations fall in the range of 50 to 150 days. One might estimate the mean to be about 90 days.

2.3. Box plots

A third method to look at the distribution of data is with box plots. Box plots summary features are based on ranks rather than sums. This makes these displays relatively less susceptible to the influence of extreme values. Looking at the data from Venezuela (Figure 3), one can see that several observations were made of extended days to first service. By adding parity to the box plot (Figure 4), we can divide the data into sub-populations and see that most of the cows with extended days from calving to first breeding are in their first lactation.

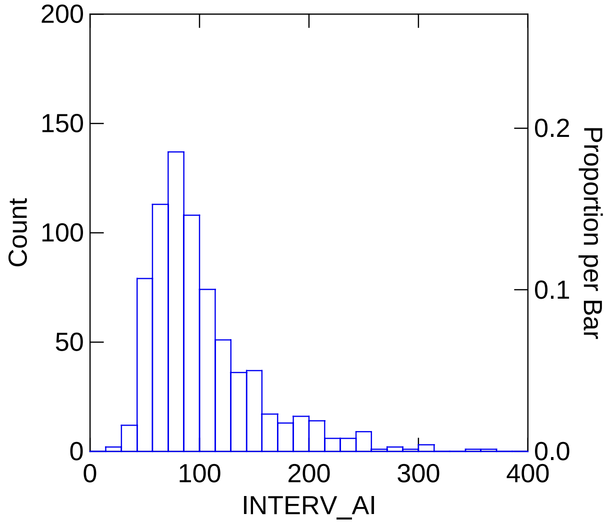

FIG.2. Histogram of interval to AI

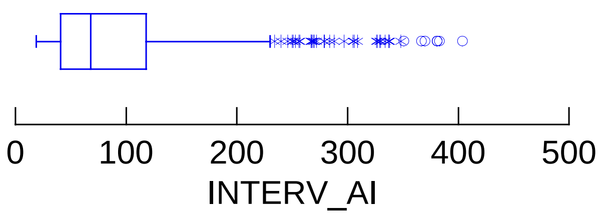

FIG.3. Box plot of interval to AI

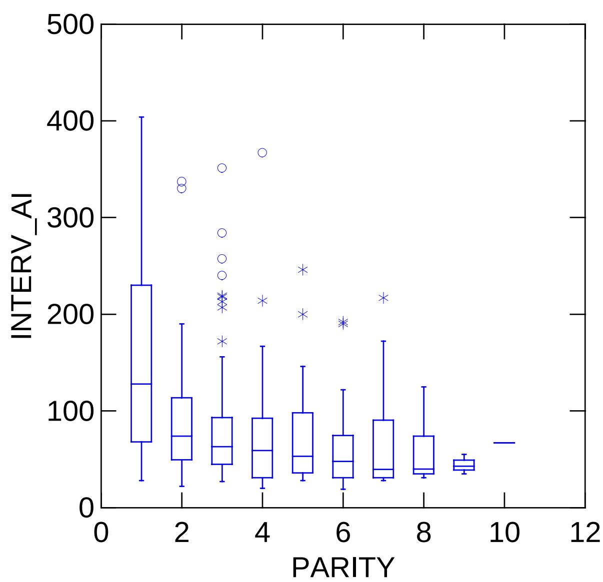

FIG. 4. Box plot of parity versus interval to AI

2.4. Descriptive statistics

Descriptive statistics are the last analysis of the initial or exploratory phase. As the name implies, they describe or summarize the data. Descriptive statistics provide as much information as possible about the sample population.

	INTERV_AI	MILK_PROD
N of cases	498	485
Minimum	19.000	1.900
Maximum	404.000	19.000
Median	68.000	7.700
Mean	94.880	8.053
95% CI Upper	101.693	8.325
95% CI Lower	88.066	7.781
Std. Error	3.468	0.139
Standard Dev	77.391	3.051
C.V.	0.816	0.379

FIG. 5. Descriptive statistics of interval to AI and milk production

Looking at the descriptive statistics in Figure 5, we notice that the measures of central tendency (mean and median) are divergent. This suggests that the population is skewed. We also notice that the co-efficient of variation is large. The confidence intervals focus on the size and credibility of an effect.

3. RELATIONSHIPS BETWEEN VARIABLES

3.1. Scatter plots

To explore and understand the data more we begin to look for relationships between variables. Scatter Plots are useful because the data is represented visually and trends or relationships may be indicated by the pattern displayed. In addition, unusual values may be revealed.

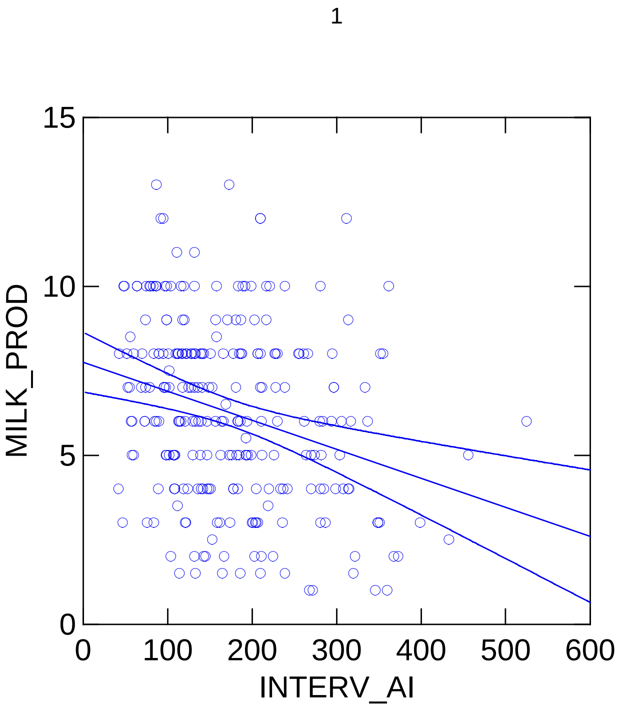

FIG. 6. Scatter plot of milk production versus interval to AI

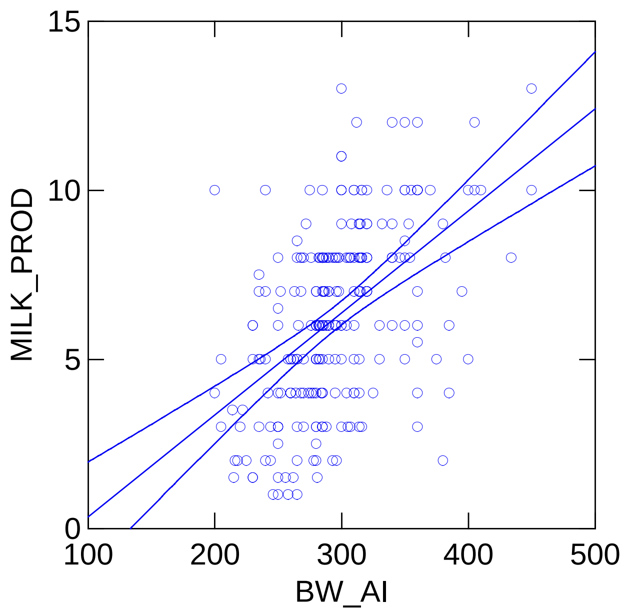

FIG. 7. Scatter plot of milk production versus body weight at AI

Inspecting the scatter graph in Figure 6 we observe a bivariate scatter plot of MILK_PROD versus INTERV_AI at 1st service with a line of best fit. The slope of the line may be an indication of the relationship of the variables. In this case it appears that as the interval from calving to 1st service increases the amount of milk produced decreases. The second plot in Figure 7 indicates that as body weight at AI increases so does milk production.

3.2. Correlation

Next one can assess the strength of this visual relationship using correlation. Correlation computes how well different variables are associated or related using a Pearson Correlation matrix as demonstrated in Figure 8. However to assess whether these correlations are significant you would add probability statistics. One can choose the Bonferroni probability option that allows for multiple testing. The correlation between body weight at AI and milk production is significant at p =.000 and interval from calving to AI is not significant at p =.116. These preliminary results can now be further examined using regression to see if an explanatory model can be formulated.

3.3. Multiple regression

Multiple regression tests the validity of a statement whereby a number of independent variables (interval AI, body weight at AI, distance to AI center, and total land of farm) attempt to explain the variability in the dependent variable (in this case milk production). The independent variables are specified using the results of the correlation and your biological knowledge.

Select 1st service only

Pearson Correlation matrix: milk_prod, bcs_ai, bw_ai, interv_ai, int_ht_ai

Pearson correlation matrix

	MILK_PROD	BCS_AI	BW_AI	INTERV_AI	INT_HT_AI
MILK_PROD	1.000				
BCS_AI	0.393	1.000			
BW_AI	0.422	0.477	1.000		
INTERV_AI	-0.249	-0.089	-0.158	1.000	
INT_HT_AI	-0.015	0.124	0.119	0.113	1.000

Bartlett Chi-square statistic: 61.607 DF=10 Prob= 0.000

Matrix of Bonferroni Probabilities

	MILK_PROD	BCS_AI	BW_AI	INTERV_AI	INT_HT_AI
MILK_PROD	0.0				
BCS_AI	0.000	0.0			
BW_AI	0.000	0.000	0.0		
INTERV_AI	0.116	1.000	1.000	0.0	
INT_HT_AI	1.000	1.000	1.000	1.000	0.0

Number of observations: 102

FIG.8. Pearson Correlation

Multiple Regression Milk_prod=dependent interv_ai, dist_ai, land_totl, and bw_ai=independent:

Dep Var: MILK_PROD N: 102 Multiple R: 0.483 Squared multiple R: 0.234

Adjusted squared multiple R: 0.202 Standard error of estimate: 2.372

Effect	Coefficient	Std Error	Std Coef	Tolerance	t	P(2 Tail)
CONSTANT	1.018	1.607	0.0	.	0.634	0.528
INTERV_AI	-0.004	0.002	-0.166	0.948	-1.819	0.072
DIST_AI	-0.188	0.115	-0.150	0.939	-1.641	0.104
LAND_TOTL	0.026	0.078	0.030	0.958	0.335	0.738
BW_AI	0.022	0.005	0.421	0.918	4.540	0.000

Analysis of Variance

Source	Sum-of-Squares	DF	Mean-Square	F-Ratio	P
Regression	166.542	4	41.635	7.397	0.000
Residual	545.978	97	5.629		

FIG. 9. Multiple regression

The regression statistics are displayed in Figure 9 for data from Sri Lanka. The squared multiple R is .234, indicating the independent variables are explaining about 23% of the variation in milk production. The overall regression model is significant at p = 0.000 and the specific independent variables responsible for this appear to be INTERV_AI and BW_AI which are significant at p = 0.072 and 0.00.

Residuals are plotted in the Systat Graph window when a Regression analysis is done. Ideally one should not be able to detect any pattern in the data plotted in the residuals graph. The points should be scattered randomly above and below the zero line. To assess how well the data can be described using a linear regression model, one can look at the residual plot provided in Figure 10. The residual plot shows that the residuals are small (close to 0.0 dashed line) and there is no systematic pattern in the residuals: the points seem to be scattered without any tendency to drift up or down or to curve or fan out.

Milk_prod=dependent interv_ai, tech_age, land_totl, and bsc_ai=independent:

```
Dep Var: MILK_PROD  N: 125  Multiple R: 0.534  Squared multiple R: 0.285

Adjusted squared multiple R: 0.261  Standard error of estimate: 6.505

Effect      Coefficient  Std Error   Std Coef Tolerance   t  P(2 Tail)

CONSTANT      32.528     5.474     0.0     .     5.943  0.000
INTERV_AI      -0.027     0.008     -0.293   0.726  -3.235  0.002
TECH_AGE      -0.037     0.076    -0.049   0.575  -0.486  0.628
LAND_TOTL      0.017     0.006     0.291   0.643  3.025  0.003
BCS_AI       -2.435    1.022    -0.198   0.863  -2.382  0.019

               Analysis of Variance

Source       Sum-of-Squares  DF Mean-Square   F-Ratio    P

Regression       2025.149   4   506.287   11.964    0.000
Residual        5078.237  120    42.319
-------------------------------------------------------------------------------

*** WARNING ***
Case      20 has large leverage  (Leverage =    0.243)
Case      22 has large leverage  (Leverage =    0.230)

Durbin-Watson D Statistic   1.246
First Order Autocorrelation  0.373
```

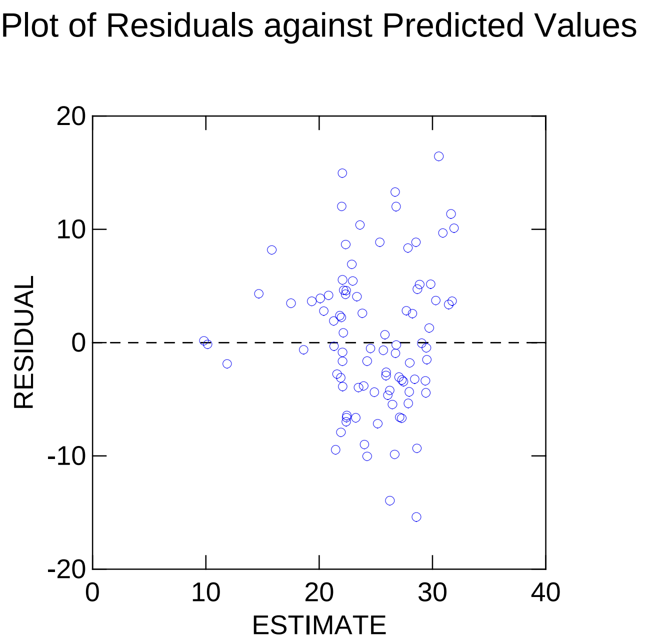

FIG. 10. Multiple regression and plot of residuals

Multivariate regression indicates INTERV_AI (as the interval decreases milk yield increases). Additionally, LAND_TOTL, and BCS_AI have significant influence on the variation in MILK_PROD and are responsible for 29% of the variation (including TECH_AGE, which was not significant). However, it is noted that the Residual plot indicates that INTERV_AI should be transformed with logs because there appears to be a systematic pattern of increase as the estimate gets larger.

After transforming INTERV_AI and redoing the analysis substituting the log of INTERV_AI, the analysis and Residuals Plot given in Figure 11 is observed:

```
Dep Var: MILK_PROD  N: 125  Multiple R: 0.563  Squared multiple R: 0.317

Adjusted squared multiple R: 0.294  Standard error of estimate: 6.360

Effect     Coefficient  Std Error   Std Coef Tolerance   t  P(2 Tail)

CONSTANT      55.045    8.749    0.0    .    6.292  0.000
TECH_AGE      -0.049    0.074    -0.066   0.593  -0.671  0.503
LAND_TOTL      0.015    0.006    0.255   0.631  2.685  0.008
BCS_AI       -2.351    0.998    -0.191   0.866  -2.356  0.020
LOGINTV       -5.469    1.346    -0.356   0.740  -4.063  0.000

               Analysis of Variance

Source       Sum-of-Squares  DF Mean-Square   F-Ratio    P

Regression       2249.913   4   562.478   13.907    0.000
Residual        4853.473  120    40.446
-------------------------------------------------------------------------------


Durbin-Watson D Statistic   1.319
First Order Autocorrelation  0.338
```

Plot of Residuals against Predicted Values

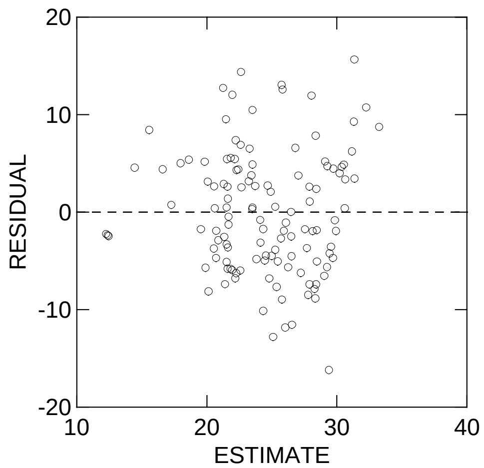

FIG. 11. Multiple regression and plot of residuals after transformation

The transformation of the INTERV_AI with logs resulted in a regression that had a more random pattern in the residuals plot.

3.4. Analysis of variance

A statistical method to analyze data using a continuous dependent variable (LOGINTV) and a categorical independent variable (CALV_MONTH) is analysis of variance (ANOVA). The analysis is concerned with comparing the number of days to first service means both between and within the various months of calving. Data from Cuba is presented in Figure 12.

The ANOVA analysis indicates that there is a significant difference in days to first breeding by calving month. However, the month-to-month fluctuation is quite extensive resulting in no consistent pattern of reproductive management, which may have some influence on the low conception rates.

```
Effects coding used for categorical variables in model.

Categorical values encountered during processing are:
CALV_MONTH (12 levels)
     1,     2,     3,     4,     5,     6,     7,
     8,     9,    10,    11,    12

Dep Var: LOGINTV  N: 254  Multiple R: 0.454  Squared multiple R: 0.206


               Analysis of Variance

Source       Sum-of-Squares  DF Mean-Square   F-Ratio    P

CALV_MONTH        22.978  11    2.089    5.697    0.000

Error           88.732 242    0.367

-------------------------------------------------------------------------------

*** WARNING ***
Case      6 is an outlier    (Studentized Residual =    -3.773)

Durbin-Watson D Statistic   1.559
First Order Autocorrelation  0.206
```

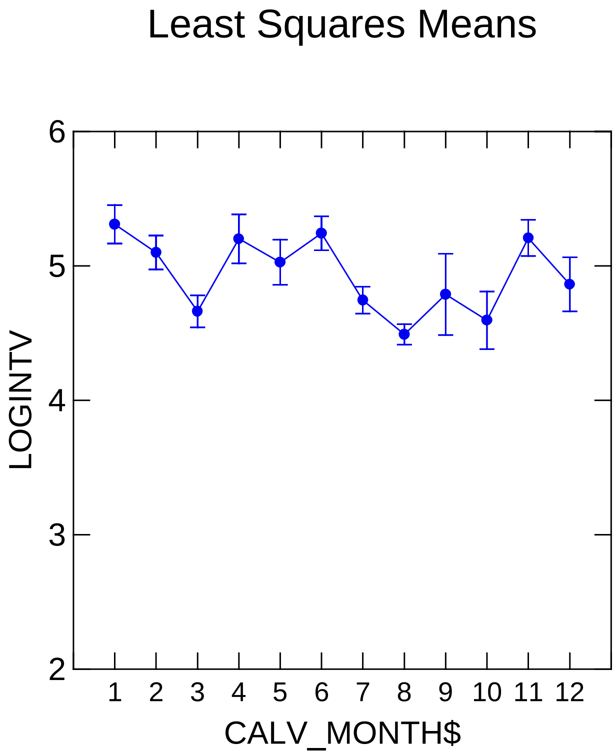

FIG. 12. Analysis of variance and least squares means

3.5. Frequency tables

Frequency tables allow you to test the hypothesis between two discrete variables. For example, there is no association between the AI technician and pregnancy rates. The actual value of the chi-square statistic tells you only about the independence of the variables. It provides no information about the strength, direction, or pattern of the association. However in the context of exploration to uncover areas of an AI program that should be reassessed it can be very useful as the examples in Figures 13 and 14 will demonstrate.

```
Frequencies
 TECH_NAME$ (rows) by PREGNANCY (columns)

          0     1    Total
      +---------------------+
  ACERBI |    16     0 |    16
   BAJIN |     7    21 |    28
 BRANBILLA |    17     0 |    17
   Diaz |    1     0 |     1
 GONZALEZ |    32     0 |    32
  LUCIANO |     4     0 |     4
 NATALINI |     3     0 |     3
  PARENTI |    66     0 |    66
  PIEDRA |    6     0 |     6
 SCHURRER |    25    20 |    45
      +---------------------+
 Total      177    41     218

Percents of total count
 TECH_NAME$ (rows) by PREGNANCY (columns)

          0     1    Total   N
      +---------------------+
  ACERBI |  7.339   0.0  |   7.339    16
   BAJIN |  3.211   9.633 |  12.844    28
 BRANBILLA |  7.798   0.0  |   7.798    17
   Diaz |  0.459   0.0  |   0.459     1
 GONZALEZ |  14.679   0.0  |  14.679    32
  LUCIANO |  1.835   0.0  |   1.835     4
 NATALINI |  1.376   0.0  |   1.376     3
  PARENTI |  30.275   0.0  |  30.275    66
  PIEDRA |  2.752   0.0  |   2.752     6
 SCHURRER |  11.468   9.174 |  20.642    45
      +---------------------+
 Total     81.193  18.807   100.000
 N        177    41          218

Row percents
 TECH_NAME$ (rows) by PREGNANCY (columns)

          0     1    Total   N
      +---------------------+
  ACERBI | 100.000   0.0  |  100.000    16
   BAJIN |  25.000  75.000 |  100.000    28
 BRANBILLA | 100.000   0.0  |  100.000    17
   Diaz | 100.000   0.0  |  100.000     1
 GONZALEZ | 100.000   0.0  |  100.000    32
  LUCIANO | 100.000   0.0  |  100.000     4
 NATALINI | 100.000   0.0  |  100.000     3
  PARENTI | 100.000   0.0  |  100.000    66
  PIEDRA | 100.000   0.0  |  100.000     6
 SCHURRER |  55.556  44.444 |  100.000    45
      +---------------------+
 Total     81.193  18.807   100.000
 N        177    41          218

WARNING: More than one-fifth of fitted cells are sparse (frequency < 5).
     Significance tests computed on this table are suspect.
Test statistic             Value     DF      Prob
 Pearson Chi-square         110.856    9.000     0.000
```

FIG. 13. Frequency tables, effect of technician

However sometimes solutions to problems are simple and in this case it appears that the AI service needs a training program for inseminators with limited experience as 2/3 of the inseminations were not successful that were performed by inseminators with less than 12 years of experience. In both cases Pearson Chi-square is significant.

Frequency tables associating INT_HT and pregnancy (Figure 15) raised some interesting questions in the Indonesian data. On the large scale farm which has a herd of 6000 beef cows the interval from heat to AI was much longer (range = 10–18 hours) while in the small-holder farms the interval was shorter (1–3 hours). On the large scale farm most cows became pregnant when bred 10 hours after heat was noticed and the proportional number of pregnancies decreased as cows were bred 18 hours after heat was noticed. Pearson Chi-square was significant. Whereas, on the small-holder farms (Figure 16) most cows became pregnant when bred 2 hours after heat was noticed and the proportional number of pregnancies remained about the same at 1 and 3 hours post-heat. Pearson Chi-square was not significant.

```
Frequencies
 YRS_EXPER (rows) by PREGNANCY (columns)

       0   1   Total
    +---------------+
 1.082 |   6   0 |   6
 2.581 |   1   0 |   1
 4.389 |  16   0 |   16
 5.227 |  66   0 |   66
 5.471 |  32   0 |   32
 8.584 |   4   0 |   4
 9.584 |  17   0 |   17
 11.586 |   3   0 |   3
 22.345 |  25   20 |   45
 25.378 |   7   21 |   28
    +---------------+
 Total   177   41    218



Percents of total count
 YRS_EXPER (rows) by PREGNANCY (columns)

        0    1   Total  N
     +-----------------+
  1.082 | 2.752  0.0  |  2.752    6
  2.581 | 0.459  0.0  |  0.459    1
  4.389 | 7.339  0.0  |  7.339   16
  5.227 | 30.275  0.0  | 30.275   66
  5.471 | 14.679  0.0  | 14.679   32
  8.584 | 1.835  0.0  |  1.835    4
  9.584 | 7.798  0.0  |  7.798   17
 11.586 | 1.376  0.0  |  1.376    3
 22.345 | 11.468  9.174 | 20.642   45
 25.378 | 3.211  9.633 | 12.844   28
     +-----------------+
 Total   81.193 18.807  100.000
 N      177   41        218



Row percents
 YRS_EXPER (rows) by PREGNANCY (columns)

        0    1   Total  N
     +-----------------+
  1.082 |100.000  0.0  | 100.000    6
  2.581 |100.000  0.0  | 100.000    1
  4.389 |100.000  0.0  | 100.000   16
  5.227 |100.000  0.0  | 100.000   66
  5.471 |100.000  0.0  | 100.000   32
  8.584 |100.000  0.0  | 100.000    4
  9.584 |100.000  0.0  | 100.000   17
 11.586 |100.000  0.0  | 100.000    3
 22.345 | 55.556 44.444 | 100.000   45
 25.378 | 25.000 75.000 | 100.000   28
     +-----------------+
 Total   81.193 18.807  100.000
 N      177   41        218



WARNING: More than one-fifth of fitted cells are sparse (frequency < 5).
     Significance tests computed on this table are suspect.
Test statistic             Value     DF      Prob
 Pearson Chi-square         110.856    9.000     0.000
```

FIG. 14. Frequency tables, effect of experience of technician

```
Frequencies
 INT_HT_AI (rows) by PREGNANCY (columns)

      0   1  Total
    +-------------+
   6 |  2   0 |   2
   7 |  2   1 |   3
  10 |  43  35 |  78
  14 |  34  13 |  47
  18 |  82   6 |  88
    +-------------+
 Total  163  55   218

Test statistic             Value     DF      Prob
 Pearson Chi-square         32.692    4.000     0.000
```

FIG. 15. Frequency tables, effect of interval from heat to AI in a large farm

```
Frequencies
 INT_HT_AI (rows) by PREGNANCY (columns)

0   1  Total
    +-------------+
   1 |  29  16 |  45
   2 |  30  37 |  67
   3 | 197  133 |  330
   4 |  13  10 |  23
   5 |  4   2 |   6
   6 |  4   0 |   4
   8 |  6   1 |   7
   9 |  1   0 |   1
  10 |  1   0 |   1
  12 |  4   1 |   5
    +-------------+
 Total  289  200   489

Test statistic             Value     DF      Prob
 Pearson Chi-square         13.580    9.000     0.138
```

FIG. 16. Frequency tables, effect of interval from heat to AI in small holder farms

4. CONCLUSION

By using GAIDA the researcher is assisted in summarizing and understanding relationships in their data. In addition, GAIDA will help guide the Statistician in the initial and repeated analyses as well as help identify specific areas to concentrate future research and interventions.

REFERENCES

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[2] GARCIA, M., PERERA, O., Artificial Insemination Database Application (AIDA), User Manual, Animal Production and Health Section, IAEA, Vienna (1996).

A STRATEGY FOR ESTABLISHING DIAGNOSTIC AND RELATED SERVICES TO DAIRY FARMERS IN DEVELOPING COUNTRIES BASED ON RADIOIMMUNOASSAY OF PROGESTERONE IN MILK

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Abstract

A STRATEGY FOR ESTABLISHING DIAGNOSTIC AND RELATED SERVICES TO DAIRY FARMERS IN DEVELOPING COUNTRIES BASED ON RADIOIMMUNOASSAY OF PROGESTERONE IN MILK.

The radioimmunoassay (RIA) for progesterone in milk samples collected from cattle has been used for monitoring ovarian activity, diagnosis of pregnancy and non-pregnancy, assessment of the accuracy of oestrus detection and for surveying efficiency of artificial insemination services. The establishment of a service to dairy farmers in developing countries based on this technique has not been previously reported but there are clear potential benefits in such a service. A strategy was therefore developed for the establishment of diagnostic and related services to dairy farmers in Morocco on a pilot basis, using RIA of progesterone in milk for possible adoption as a model for other developing countries.

1. INTRODUCTION

Radioimmunoassay (RIA) of progesterone in milk has been used extensively for investigations of reproductive processes in cattle in many countries [1 to 20]. In developing countries monitoring ovarian activity, diagnosing non-pregnancy and pregnancy, assessing the accuracy of oestrus detection, surveying the efficiency of Artificial Insemination (AI) services, and studying the effectiveness of interventions such as feed supplements [21, 22] are amongst the recent uses of assaying progesterone in milk.

Well developed, cost effective veterinary and AI services and a high level of farmer education combine in many developed countries to provide a system for monitoring ovarian activity and oestrus postpartum and the diagnosis of pregnancy, non-pregnancy and infertility. In developing countries, especially where there are small farms with poor communication and accessibility, such support activities related to herd reproduction are often unavailable or too expensive. To alleviate poverty and to increase productivity on small farms it is essential to improve the efficiency of livestock production. An RIA service for milk progesterone has potential for providing information to farmers in developing countries about the reproductive events in their herds as a basis for improving reproductive management and thereby the efficiency of production.

In Germany [12], Finland [11] and France [8, 23] such services have been established. No references have been found describing similar services in developing countries.

The results obtained under a Co-ordinated Research Project of the Joint FAO/IAEA Division of the International Atomic Energy Agency (IAEA), Vienna, conducted in 14 Asian and Latin American countries [this publication] have shown the value of the progesterone assay in monitoring AI services and identifying lapses in reproductive management by smallholder farmers in developing countries. The IAEA is therefore initiating programmes for introduction of this technology as a service to farmers, through a regional approach under the framework of its Technical Co-operation programme. In Africa, Morocco was selected as a starting point for the development of an appropriate model which could be followed also by other countries. Prerequisites for such services were considered to be the presence of a well-organised and sustainable AI service and a willingness on the part of Government and the industry to support the new initiative. These conditions were met in Morocco, which was chosen for an initial trial and as a model for African countries. RIA of progesterone in milk had also been used previously in Morocco for some investigation work [24, 25, 26].

The objective of the present work was to develop a strategy for establishing a service to farmers in Morocco based on the RIA of progesterone in milk samples.

2. METHODS

Initially, a planning meeting was organized by the IAEA in Rabat (Perera), at which staff of the Ministry of Agriculture (Manar) and the National Association of Cattle Breeders participated, together with livestock personnel from four selected African countries. Following site visits to the main AI centres and discussions, a project plan was developed for support by IAEA under the framework of the Regional Co-operative Agreement for Africa (AFRA), during 1999–2000. The major part of the work was conducted during the planning meeting, meetings with officials and inspection of AI facilities in Morocco (Perera, Manar). Thereafter, a two-week expert mission was carried out in Morocco in December 1998 (Galloway, Manar), followed by a two-day discussion session at the IAEA headquarters in Vienna (Galloway, Perera).

Observation, interview, discussion and the review of Government documentation were used to assess the status of the cattle industry and AI, to define the constraints to further development of cattle production and AI services and to determine willingness of government and industry to establish and sustain a service. The functions of AI centres and technicians were assessed against documented good practice [27–41] and experience of it. Cost effectiveness could not be evaluated until more preliminary work was carried out. This aspect was therefore dealt with by proposing it as part of a research project (Section 3.2.4.7.)

3. RESULTS AND STRATEGY

3.1. Assessment

3.1.1. The cattle industry in Morocco

Milk production has been the first priority with the objective of making the country self sufficient in dairy produce. The programme of development set out by the Ministry of Agriculture and Rural and Maritime Industry Development has included import of improved breeds, improvement of forage crops, disease control, marketing and the support and encouragement of professional organisations within the industry. The programme aims at an increase in milk production of 6.3% per annum (present rate 2.3%) to meet the estimated demand of 3.8 million tonnes in 2020. The proportion of cattle of improved pure dairy breeds (Pie Noire, Holstein) and their crosses increased from 10% of the population in 1970 to 37% in 1996. The aim is to increase this to 70%, principally through AI.

The Ministry of Agriculture provides services to the industry in the areas of health and hygiene, animal breeding, management and production through regional Ministry offices (ORMVA, Offices Régionaux de Mise en Valeur Agricole). There is a progressive policy of transferring AI services and responsibility for milk recording and milk quality control to the private sector in the form of the strong and active co-operative organisations, specifically the National Association of Cattle Breeders (ANEB, Association Nationale des Eleveurs de Bovins Races Pures, created in 1990) and the milk co-operatives. Eighty five percent of farms are small with 3 – 4 cows. These plus larger farms of around 20 cows are organised to deliver milk to collection centres which serve 30 to 70 farms each. The centres are managed in a co-operative system which sell milk to the milk factories. This system provided the infrastructure necessary for the development of AI.

3.1.2. AI services in Morocco

There are two AI centres in Morocco, Ain Jemaa established in 1968, and Fouarat established in 1976. Both produce and dispatch frozen semen to areas throughout Morocco and train inseminators. They import animals from Europe and semen from Canada and Europe and have begun to undertake progeny testing locally. They also have training courses for young farmers.

At the provincial level the Centres are supported by regional sub-centres for AI, supervised by the Provincial Livestock Services (DPA, Services d'Elevage des Directions Provinciales d'Agriculture) and the ORMVA as well as the milk co-operatives and the ANEB organisation. ANEB has now created 16 regions, each with a regional office and encompassing a total of 64 AI circuits of approximately 100 km in length and a minimum of 2000 cows eligible for insemination. An insemination technician travels the circuit each day equipped with a vehicle, liquid nitrogen tank and insemination equipment. Farms close to the roads of the circuit are visited. Many farmers bring their cows on foot to the roadside to meet the inseminator. These circuits are termed the "mobile circuits" (Postes Mobiles). Approximately 10% of the total services are provided at "fixed stations" (Postes Fixes) where the inseminator is stationary and to which farmers bring their animals.

In 1996 ANEB and the milk co-operatives had taken the responsibility for 85% of the AI circuits. In 1997 the number of inseminations carried out was 128 000 which is a three fold increase since 1990 when privatisation began. They provide assistance and services to farmers in genetic improvement, AI, nutrition, administration and record keeping. ANEB is very active and with the co-operation of the Government it is gradually taking over the responsibility for a number of service areas in the dairy industry. Recently some ANEB regional associations have begun to establish laboratories for milk quality control.

The results of AI were difficult to determine. Estimates from the available records suggest that calving interval is 410 days and the services per conception index are 2.4. These figures probably represent the better and bigger farms which have contributed most to the records. Overall estimates await improvement of record keeping and analysis of records.

Similarly, nutrition, management, especially heat detection and infectious disease will certainly be contributing to poor reproductive efficiency to AI on farms but their relative contribution is not clear at present. Private veterinary services are expensive and not readily available, especially to the small farmers and the involvement of private veterinarians in routine reproductive efficiency control appears to be minimal.

3.1.3. The AI Centres and insemination technicians

The AI centres had a high standard of professional and technical competence and management (Fig.1). Minor criticism could be made concerning the handling of bulls before and at service. The main area requiring improvement was in the exploitation of data collected from the centres, the farmers and the AI technicians for the benefit of the industry.

The AI technicians were impressive with a very high standard of technical competence and conscientiousness for maintaining a high standard and a high conception rate (Fig.2). Some, but not all technicians have telephones at home, none have mobile phones. In any case relatively few small farmers have telephones.

The inseminators relate to the farmer community very well. They attend regular refresher courses. Their main problems are in assisting the farmer with detection of oestrus, early repeat inseminations, early pregnancy diagnosis, and offering advice about how to improve reproductive efficiency under the environmental and economic circumstances prevailing. Too many cows had lost ear tags. Too many cows were in condition score of 2.5 or lower (0–5 scale). Some records arriving at the ANEB centre were not legible.

3.1.4. Constraints to improvement and development

The constraints to the development of the dairy industry and to AI services were associated with identification of cattle, reliability of data, education of farmers, nutrition and disease control, availability of finance for improvements in management and nutrition, especially for small farmers, transport and communication and lower than desirable membership of co-operatives. These are widespread problems in developing countries and some activities of both Government and co-operatives are directed towards improvement in Morocco.

The other main constraint was the difficulty in using the present data collection and analysis system to obtain a meaningful basis for planning improvements and innovations. Improved definition of inefficiencies at the cow, herd, farmer, inseminator, bull and centre levels is needed. The information about AI is good as far as it goes. But it is insufficient and difficult to exploit for the benefit of the centres, the AI technicians and the farmers (Figs.1 and 2).

3.2. Strategy

The strategy is set out in terms of the recommendations that were made for the project.

3.2.1 Objective of the service

This was defined as the provision of a cost effective RIA service on milk samples to farmers to give them information about AI timing, cyclicity, non-pregnancy and pregnancy. The main components of the service were considered to be equipment and training, identification of cows, collection of milk samples, labelling, preservation and transport of samples, washing and re-use of sample tubes, routine RIA, feedback to farmers of results and their interpretation, follow up with professional advice and assistance, and transfer of financial support from research and development funds to a sustainable, user-pays system.

3.2.2. National AI Development Committee and staffing

It was considered necessary that a National AI Development Committee (NAIDC) be formed, to be responsible for the implementation and monitoring of the project in Morocco and for later activities associated with the wider AFRA project. Composition of the committee was proposed as follows: Chairman, Head of the Ministry for Agriculture, Co-Chairman, the official responsible for AI in ANEB (CANEB), AI co-ordinator (AIC) from the Ministry of Agriculture, the two heads of the AI Centres, and the Head of the RIA laboratory and professor in animal reproduction at the University. Essential staff for implementing the project were considered to be (a) two people to plan and implement the project and to take part in training programmes (CANEB and AIC), (b) the heads of each AI centre and one deputy each, a technical person at each centre responsible for the day to day running of the service, (c) two field veterinarians familiar with AI centres, AI circuits and farms to advise on extension and training and provide clinical backup services, (d) two people from outlying centres likely to be involved in running laboratories in the future, (e) two AI technicians to be involved in training others in all aspects of the project, and (f) the professor from the University School of Agriculture and Veterinary Science responsible for research projects and some training and his technical assistant competent in RIA.

3.2.3. Establishing the laboratories and equipment

The existing laboratory spaces at the two AI centres were examined and the refurbishments necessary for them to function as RIA laboratories were identified. Equipment and disposable materials were to be obtained where possible, from local suppliers and with service contracts for major items. The major equipment included the Gamma counter with on line data processing using a computer which also had the capacity to run a database application for field and laboratory data analysis, a refrigerated centrifuge, a refrigerator with freezer, an analytical balance and a radioactive contamination detector. Other items included laboratory instruments such as micropipettes, hand-held repeater pipette multi dispenser unit, magnetic stirrer, vortex mixer, pH meter and foam decantation racks. Sample tubes, ear tags and ear tag applicators, labels and forms for data collection were to be obtained locally.

BULLS Health and Well-being +++	BULLS Genetic Quality +++	BULLS Housing +++
BULLS Feeding +++	BULLS Handling before / during collection ++**(+)**	COLLECTION TECHNIQUE +++
FACILITIES and EQUIPMENT +++	HANDLING and ASSESSING SEMEN +++	PROCESSING TECHNIQUES +++
SEMEN HANDLING and DESPATCH +++	RECORD KEEPING +++	EXPLOITING RECORDS and RESULTS **0**

FIG. 1. Assessment of Artificial Insemination Centres at Ain Jemaa and Fouarat in Morocco

Scoring system: Poor = 0, Fair = +, Good = ++, Very Good = +++.

Reasons for not scoring +++: *Bulls, handling before and during collection* - halter and nose ring is preferred; early and continuous training helps control of bulls; more sexual preparation and stimulation before collection is preferred; *Exploiting Records and results* - analysis of records was only undertaken to know how many AI were carried out; problems of obtaining reliable results from many farms and low level of veterinary control of reproductive efficiency prevent analysis of heat detection efficiency, and performance of individual cows, herds, inseminators, semen batches and bulls, embryonic death, abortion, still births and normal births.

Heat Detection Farmer / AI technician ++**?**	Timing AI Once per day service ++**?**	Training and Refresher courses +++
Semen handling pre-thaw +++	Thawing +++	Semen handling post thaw +++
Insemination Technique +++	Record keeping +++	Results & Exploitation of results for improvement +

FIG.2 Assessment of artificial insemination technicians and procedures in AI circuits of Doukkala and Settat, Morocco

Scoring system: Poor = 0, Fair = +, Good = ++, Very Good = +++.

Reasons for not scoring +++: *Heat detection, and timing of AI* could not be thoroughly assessed since cows were presented at the roadside for AI (Circuit Doukkala) on the basis of mucus appearance and accepted after inspection of the vulva by the technician. There was considerable confidence in this system but there was no way of checking its efficiency. A positive feature was the *good extension service and the training courses* that are held in Morocco for young farmers. These would be having a beneficial effect on the efficiency of AI. *Results and exploitation of results*. Record keeping was meticulous as far as it could be. Too many cows had lost ear tags. Cows were not always brought back for pregnancy diagnosis and when they were it was generally between 3 and 5 months after AI. Detailed, reliable and early results of AI were not sufficiently available to the inseminator to form the basis of interventions.

3.2.4. Establishing the service to farmers

3.2.4.1. Exploratory work and development of techniques

There were facilities and expertise already in Morocco to begin immediately to collect some milk samples and assay them for progesterone. This activity was to be based at the AI centres with progesterone being assayed at the central University RIA laboratory. It was recommended that the team of people responsible for undertaking the work and for training others, start immediately to explore ways of collecting samples, labelling, washing and re-use of sample tubes, transport, assaying and providing feedback to farmers. It was to be understood by inseminators and farmers that this is a phase for developing techniques and their co-operation should be sought on that basis. Farmers for example in the Doukkala co-operative were keen to participate. This exploratory work could be planned as a mini-trial and survey as a forerunner to the next step.

3.2.4.2. Trial and survey based on three milk samples

It was recommended that an initial survey be planned to obtain complete data on at least 500 cows at each AI centre based on three milk samples and where possible clinical follow up. The milk samples should be collected on day 0 (day of insemination), day 10–12 and day 22–24.

3.2.4.3. Diagnosis of non-pregnancy based on one or two milk samples

Starting at the same time as the survey it was considered appropriate to offer farmers a trial service for diagnosis of non-pregnancy on a cost-free basis for one year. The service could be based on one sample of milk submitted at day 22–24 after AI. It is preferable and adds information if the AI technician takes a sample from the cow at the time of AI and the result is based on the two samples. Milk samples would be accepted from cows with a record of AI 22–24 days previously and which were clearly labelled with the cow's identification, the date of AI and the date of sampling.

3.2.4.4. Pilot project for outlying centres

It was recommended that activities specified in 3.2.4.2.and 3.2.4.3. be initiated also at one outlying centre (Doukkala), to demonstrate the cost effectiveness and sustainability of a service to farmers. This is likely to be able to start in the second year of the programme and would be of benefit by the experience gained in the first year. The aim is to charge the farmers for the service from the outset, with perhaps some incentive for small farmers to participate.

3.2.4.5. Long-term development

Other outlying centres should adopt the progesterone assay service to farmers when its cost effectiveness has been demonstrated and as numbers of samples increase to the point where the two AI centres and the single regional centre can no longer cope with them.

3.2.4.6. Training

Specific training activities needed in support of the project were identified as scientific visits to France for the AIC and CANEB and the heads of the two AI Centres to study the use of the RIA as a service to farmers, and a training course for the four technical people selected to be responsible for the assay and the service at the two AI centres. Ideally the latter would have three components: i) training at the University in all aspects of RIA and the use and care of equipment; ii) training under supervision at the AI centres using the new equipment as soon as it is installed; iii) the National Training Workshop recommended below.

It was recommended that a National Training Workshop on "Establishing a sustainable service to farmers for diagnosis of non-pregnancy and infertility in dairy cattle" be held. All staff, some of them functioning as instructors, and others including farmer representatives and AI technician representatives should be involved. The workshop should live up to its name and not be seen as a lecture forum for "experts" to tell people how to do things or expound on irrelevant topics. The Committee should plan a draft schedule for the workshop to be modified in the light of experience. Contributors should be drawn as far as possible from Moroccan sources. This will give experience to people who will later be involved in similar workshops in the AFRA programme.

Subjects suggested for the workshop included physiology of reproduction, the constraints to AI development listed in 3.1.4. and each step in the process from proper collection of the milk samples to the mechanism of feedback of results and interpretation to farmers. Discussion of research priorities and postgraduate training in the context of the service was considered important.

As a general policy, it was considered appropriate that AI technicians be given training in all aspects of the project in their regular refresher courses, and that training courses for farmers be held regularly at the AI centres introducing them to the assay service and giving emphasis to the importance of identification, good records, oestrus detection, the meaning of assay results and the implication of early non-pregnancy diagnosis, in terms of management. Such training of farmers and AI technicians should begin immediately, be under the initiative of local counterparts and organised and conducted by them using local resources.

3.2.4.7. Research

The project provides a useful vehicle for postgraduate research and training. Important areas include assessing reproductive wastage in the AI Centres and circuits through the survey and assessing the economic cost/benefit ratio of the assay service under Moroccan conditions. Disease control using the infrastructure of the project as a basis could also be the subject of a postgraduate research project.

3.2.5. Addressing constraints to improvement and development

When AI was originally introduced in many countries, it resulted in a new phase of education of farmers, a new interest in record keeping and better management as a result of the better animals being produced and the perceived benefits in looking after them. The project should be used as a similar stimulus for improvement.

The project provides the opportunity to introduce new ear tags for cows if they have not already got yellow plastic ear tags. It was recommended that all cows in the survey as well as all cows submitted for milk sampling and pregnancy diagnosis should be tagged in this way. The idea was to build confidence in a standardised method of identification associated with the project.

Farmer and AI technician education associated with the project could emphasise the importance of standardised and accurate identification and the need for clear legible records and certificates, thereby building confidence in the method. Cows entering the survey and being identified for that purpose could form a nucleus for testing for infectious diseases, especially Brucellosis and Tuberculosis.

The idea of providing incentives to farmers to submit cows and milk samples properly labelled and at the correct time is attractive. Pregnancy diagnosis at no extra charge than previously was recommended for the first year. The co-operatives could consider a points system for discounts on feed supplements or services supplied by them. A calving to conception interval below 100 days could be rewarded in a similar manner, for example with discounted concentrates for the cow as she approaches the next calving. These initiatives would also make membership of the co-operatives attractive to farmers.

4. DISCUSSION

4.1. Assessment

The rationale for this project arose from the findings from an FAO/IAEA Co-ordinated Research Project where 14 participants in Asia and Latin America used the progesterone assay as a tool to monitor AI services provided to smallholder dairy farmers and thereby identify the causes for low reproductive efficiency. In order to develop a strategy for using this technique to provide a diagnostic service to farmers, Morocco was selected by IAEA as an ideal country in which to conduct a pilot project, which could be used to establish a progesterone assay service to farmers as a model for other African states. The co-operative system was well developed and progressive privatisation has helped secure the sustainability of AI and will continue to do so. Morocco has a strong incentive to improve efficiency in the cattle industry because of imminent trade liberalisation with the European Economic Community in 2000.

The reasons for selecting Morocco included the presence of a well-organised and successful AI programme with good infrastructure, training programmes and support from industry and Government. AI technology was of a high standard. Minor improvements only could be suggested, but it was considered important that the project be used to improve and develop recording and analysis of results and their exploitation for improvement in reproductive efficiency.

4.2. Strategy

The composition of the NAIDC was designed to co-ordinate the roles of the Co-operatives and the Government and to ensure that their joint efforts in improving the dairy industry continues. It was considered important that representatives of these bodies have advice available on the Committee from each aspect and level of the project.

Under-used laboratory space was available in the locations proposed for RIA laboratories. Hence refurbishment rather than construction could be recommended. Equipment for the RIA is standard, but three aspects were considered important. The first was to incorporate service contracts into the purchase price where possible. The second was to obtain items locally when feasible to ensure a back up service and to retain as much of the economic input within the country as possible. The third was that adequate training on the use of equipment be provided to more than one person at each location (3.2.4.6.).

The way to gain experience and identify difficulties in a new system with new techniques is to start doing it: hence the recommendation for exploratory work to begin immediately. Trials with new equipment should start as soon as it is installed. In this way the National Training Workshop recommended in section 3.2.4.6. would be given more of a focus and be made very relevant, dealing with real difficulties already encountered under Moroccan conditions.

The trial and survey has been an important first step in experimental work in other countries [22] and is recommended for all AFRA states. Starting in this way enables considerable information about pregnancy/non-pregnancy and infertility to be accumulated at the outset. It provides a means by which the assay service can be established and monitored, and problems can be identified and corrected.

Diagnosis of non-pregnancy is highly accurate using the RIA for progesterone at the time of the next oestrus following AI, e.g. 92.4% [11], 95% [8], 97% [24]. Records of dates are essential for interpretation. In Morocco farmers depend very much on the AI technicians for advice and assistance. It was considered important to train inseminators in all aspects of the project to assist them in this work. The two veterinarians proposed for the NAIDC would be involved in developing the way in which results were interpreted and a strategy for a back up advisory service to farmers. The heads of the two AI centres, also veterinarians, would need to be involved in these aspects as well as in training others in all aspects of the project. How to handle equivocal results under routine service conditions is an important aspect. For the service to be effective in improving reproductive efficiency it is important that the farmer receives the result within two weeks. It is also important that the farmers are educated in the significance of negative, positive and doubtful results and the managerial activities that should follow each possibility. If necessary, educated and co-operative farmers could be selected to take part initially. But the system must be made user friendly for the uneducated small farmer.

Some co-operatives in the ANEB organisation were keen to introduce the service to members and a pilot project for one of the co-operatives was proposed. This was the most appropriate place for a research project to establish the economics of the service under Moroccan conditions.

Adequate training for all concerned in the project was considered essential. Apart from formal training, the workshop proposed could have sessions devoted to short reports of "progress and problems" in the exploratory phase (3.2.4.1.) by the people engaged in the work.

The use of RIA will certainly not solve all the problems of AI but it can be used as a vehicle to introduce remedial measures. Proper identification of cows and good record keeping are important elements in making the AI service work and must be addressed. A gamma counter with on line data processing and AI data analysis capacity would provide the means to collate AI records and to exploit them to improve reproductive efficiency. The training programmes recommended will have benefits beyond the immediate project. Disease control projects could be implemented using the infrastructure of the project.

One of the most valuable assets in developing countries are young graduates interested in undertaking postgraduate training in the agricultural industries. Aspects of the project should be made vehicles for postgraduate training to Masters and Doctorate levels. Funds for training may be more readily available than funds for research projects for established staff of universities, Government and co-operatives. Other international and bilateral donors should be sought for such support.

Reasons for difficulty or failure in implementing the project must include the motivational factors that lead to development and change in a community. Consideration needs to be given to the social template, identity and status [41]. Benefits must be evident to individuals and groups involved. Where an important component of the social template is economic then the most obvious benefit is a financial one. Where improved productivity and increased profit over additional costs are clearly present farmers are likely to adopt a new procedure. Other benefits are concerned with identity and status of individuals associated with the project. Many people involved, especially employees of organisations may not gain economically from the new scheme. Hence it is necessary for individuals to be motivated by a demonstrable increase in status and upgrading of identity within the organisation and the community. This in part is achieved in improvement in job satisfaction, knowledge of involvement in something new and worthwhile. The respect from others is another component. This suggests that such a project has to be "sold" to the community in terms of its tangible and intangible benefits and the community must take it over, own it and drive it forward.

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