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Improving the Reproductive Management of Dairy Cattle Subjected to Artificial Insemination

Publication prepared under the framework of an RCA project with the technical support of the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture

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FOREWORD

Cattle and buffalo are an integral part of the mixed crop-livestock smallholder farming systems in the developing countries of the Asia-Pacific region. Apart from being a crucial source of high quality food (meat and milk), dairy farming provides employment, sustainable income and social security to millions of smallholder farmers within the region. Also, attaining food security and self-sufficiency in livestock products is a high priority development goal of most countries in this region.

The profitability of milk and meat production from cattle and buffaloes depends to a large extent on the efficiency of reproduction. Maximizing reproductive efficiency requires the matching of genotypes to the production environment, together with appropriate husbandry practices, in order to ensure that the intervals from calving to conception are short and the rates of conception to natural or artificial breeding are high. This will result in short calving intervals, yielding more lactations and calves per lifetime of each breeding cow. The outcome will be greater economic benefits to the farmers.

Artificial insemination (AI) is widely accepted as a technology that can bring about rapid genetic improvement in cattle and buffaloes. However, optimum conception rates will only be achieved if the quality of semen used is good, the insemination is done at the most appropriate time in relation to the oestrous period, and the technicians have adequate training and skills in the procedure. Although AI is widely used in many Asian countries, the above factors, together with other socio-economic considerations specific to smallholder production systems and inadequate infrastructure for the efficient delivery of AI services, have often led to poor success rates. If these constraints can be overcome, not only would the farmers and service providers benefit, but the technology would also become more widely adopted. Wider adoption of AI could then contribute to better food security and alleviation of rural poverty.

This publication contains the results obtained by Member States in the activities of an IAEA Technical Cooperation project dealing with reproduction. It will serve as a source of information for professionals, technicians and extension workers engaged in the provision of AI services, as well as a source of reference for research workers and students in livestock and veterinary sciences.

The IAEA officer responsible of this publication was P. Boettcher of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. He was assisted by O. Perera in the editing and formatting of the manuscripts.

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IMPROVING THE REPRODUCTIVE MANAGEMENT OF SMALLHOLDER DAIRY CATTLE AND THE EFFECTIVENESS OF ARTIFICIAL INSEMINATION: A SUMMARY

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1. INTRODUCTION

Economic development is progressing rapidly in Asia. One of the many consequences of this phenomenon is an increased demand for food arising from animal agriculture. This increased demand for animal products creates the possibility for a greater dispersion of economic resources, which to this point has been largely confined to urban areas. Thus, the opportunity for poverty alleviation in rural areas exists, but obstacles to this process must be removed. A high priority of the member states of the RCA (Regional Cooperative Agreement for Asia and the Pacific) is thus to support research and the adoption of technologies that can help overcome these obstacles. Ironically, population expansion, although increasing demand, can have a net negative effect on livestock farming, by restricting the amount of land available for raising animals. Therefore, one major focus on increasing production of animal products must be the increase in productivity per animal and per unit of land. Improvement of nutrition is an important strategy for improving the output of livestock production and results can be obtained in the short-term. On the other hand, selective breeding is a highly effective and sustainable approach for increasing animal productivity in the long-term. Reproductive technologies such as artificial insemination (AI) allow single animals to have multiple progeny, reducing the number of parent animals required and allowing for significant increases in the intensity of selection, and proportional increases in genetic improvement of production.

However, in order to benefit from the advantages of AI, farmers must detect the oestrus periods of their cows accurately, ensure that insemination is done at the correct time in relation to the onset of oestrus and detect any cows that later return to oestrus, so that they can be re-inseminated without delay. Even when these conditions are satisfied, optimum conception rates (CRs) will only be achieved if the quality of semen used is good and the AI technicians have adequate training and skills in the procedures for handling semen and performing inseminations.

Although AI is widely used in many Asian countries, the above factors together with other socio-economic considerations specific to smallholder production systems, together with inadequate infrastructure for the efficient delivery of AI services, have often resulted in poor success rates. If these constraints can be overcome, not only would the farmers and service providers benefit, but the technology would also become more widely adopted and national goals of improving livestock production will be achieved faster. This will contribute to better food security and alleviation of rural poverty.

Any attempt to improve the efficiency of AI has to be based on an understanding of the most important causes for failure under each specific production system. The traditional methods used for this rely on accurate recording and analysis of reproductive events such as oestrus, services, pregnancies and calvings. However, records are rarely kept by smallholders and, even when available, do not allow an assessment of the importance of factors such as efficiency and precision of oestrus detection by the farmers or incorrect timing of insemination.

The application of radioimmunoassay (RIA) to measure the hormone progesterone, which is produced by a transient structure called the corpus luteum in the ovaries, in samples of blood or milk collected from cows at specific times in relation to AI provides a powerful tool for studies on reproductive efficiency and AI. It can determine whether, among other things, farmers are detecting oestrus accurately, AI has been done at the correct time, or the cow has not conceived and is likely to have returned to oestrus again. The advantage of the progesterone test is that non-pregnant animals can be accurately identified at an early stage, and action taken to observe them closely for heat and to get them mated again at the correct time. Furthermore, it can be used to assess the effectiveness of AI services, to identify deficiencies and to monitor the results of interventions aimed at overcoming these deficiencies.

2. BACKGROUND AND OBJECTIVES

The regional Technical Cooperation (TC) project RAS/5/035 was initiated during the 1999–2000 biennium with the two objectives of (a) strengthening and extending the field applications of feed supplementation strategies (mainly urea multi-nutrient blocks – UMB) for ruminant livestock, and (b) applying progesterone RIA in milk for monitoring and improving the reproductive management and fertility of smallholder dairy cattle subjected to AI. This project was subsequently extended for 2001–2002 with the following additional objectives:

- Development and use of medicated and non-medicated UMB for better nutrition, and establishment of facilities and training of personnel at the pilot farms and national extension agencies in the use of block technology and RIA for improved reproductive management and early non-pregnancy diagnosis (N-PD);
- Identification, propagation and use of sources from unknown or lesser-known plants, adapted to the region, as low cost animal feeds;
- Establishment of regional capability for the production and distribution of RIA reagents; and
- Development and making available two customized database applications, AIDA (Artificial Insemination Database Application) and SPeRM (Semen Processing Records Management), for use by national livestock breeding and AI services to assist farmers in improving reproductive management.

The first two objectives were for the Animal Nutrition component and the last two for the Animal Reproduction component. Due to the long-term nature of many of the project objectives and the desire to promote sustainability, the project was extended for a final two-year period (2003–2004). This extension allowed the consolidation of the results obtained in the first four years of the project and encouraged broader transfer of the outputs to stakeholders.

Each participating Member State (MS) nominated two Project Coordinators (PCs), one each for the Animal Nutrition and Animal Reproduction/AI components, respectively.

The project commenced with an initial planning meeting in Yangon, Myanmar in January 1999. The main purposes of this meeting were to plan project activities and to train coordinators on the use of the AIDA (Artificial Insemination Database Application) database for recording, analyzing and interpreting field and laboratory data. Subsequent meetings for project review and planning took place in Kuala Lumpur, Malaysia in February 2000, and in Manila, the Philippines, in February 2001. The third project review and planning meeting was held in November 2002 in Hangzhou, The People's Republic of China.

The following Regional Training Workshops were organized for the Animal Reproduction/AI component:

- Training workshop on "Production of iodinated tracer for Self-coating RIA of progesterone", 8–12 May 2000, Bangkok, Thailand;

- Task Force meeting on "Customization of AIDA for Routine Use in AI", 2–6 April 2001, Kandy, Sri Lanka;

- Workshop of national consultants on "Evaluation of breeding bulls and semen quality control", 22–26 April 2002 Faisalabad, Pakistan;

- Training workshop on "Management and utilization of field and laboratory data for breeding support services to livestock farmers", 7–11 July 2002, Mymensingh, Bangladesh;

- Workshop for Trainers on "Cattle Fertility Management for Optimum Economic Returns", 19–23 April 2004, Ludhiana, India.

The nuclear techniques addressed in the above workshops included the use of ^{125}I-progesterone as a tracer in the RIA analyses that were used to determine whether the detection of oestrus and timing of AI had been done correctly, and for early diagnosis of non-pregnancy in cattle.

The final meeting was held in from 11–15 October 2004 in Bangkok, Thailand. The objectives of this meeting were to review the results obtained during the full period of the project, including field and laboratory work, cost-benefit analyses and in-country training and education activities. Each PC was required to prepare a written report in the form of a scientific paper, which was reviewed, technically edited and formatted for publication in the Agency TECDOC.

This TECDOC contains papers for the Animal Reproduction/AI component. The main activities that were addressed in the various papers included: (a) field surveys of reproductive performance and reproductive disorders; (b) development and use of RIA for monitoring of ovarian activity; (c) development of enzyme immunoassay for monitoring of ovarian activity; (d) the use of the AIDA software; (e) training programmes for veterinarians, AI technicians, and farmers and their effects on reproduction efficiency; (f) epidemiology of factors affecting reproduction efficiency at the farm and cow level, (g) interventions designed to improve reproduction, and h) veterinary treatment of problem breeders. Table I shows which countries reported on the different activities. The papers from the Animal Nutrition component, covering other objectives of the project, are contained in another TECDOC.

TABLE I. SUBJECTS ADDRESSED IN PAPERS FROM EACH PARTICIPATING COUNTRY

Activity	BGD	CPR	IND	INS	MAL	MYA	PAK	SRL	THA
Field surveys			X						
Radioimmunoassay	X	X	X	X	X	X	X	X	X
Enzyme immunoassay									X
Databases	X		X	X	X	X		X	
Training	X	X	X	X	X	X	X	X	
Epidemiology					X				
Interventions			X	X	X	X	X	X	
Veterinary treatment	X								

BGD = Bangldesh, CPR = China, IND = India, INS = Indonesia, MAL = Malaysia, MYA = Myanmar, PAK = Pakistan, SRL = Sri Lanka, THA = Thailand

3. SYNTHESIS OF RESULTS

A summary of the results obtained from the project is given in Tables II to IV.

TABLE II. IMPACT OF VARIOUS INTERVENTIONS ON REPRODUCTION

Country	Intervention (s)	Impact
Bangladesh	Technician training	CR[a] up by 2.9%, reduced variation
India	Sephadex filtration of semen and training	CR up by 27%
Indonesia	Improved feeding and record keeping	CR up by 20%
Malaysia	Intervention package	CR up by 20%
Myanmar	Improved semen quality and training	CR up by 20.8%
Pakistan	Technician training	CR up by 14%
Philippines	Training, improved semen quality and feeding interventions	CR up by 5%
Thailand	Technician and farmer training	CR up by 8%

[a]CR = Conception rate

TABLE III. PROBLEMS IN COWS, TREATMENTS AND RESULTS

Country	No. of animals	Problem	Treatment	Result
Bangladesh	138	Anoestrus (52%)	Vitamins ADE or GnRH[a]	Overall, 68% "cured"
		Repeat breeders (11%)	2xAI or 2xAI+GnRH	
		Endometritis (14%)	Penicillin, IU[b]	
		Non detected oestrus (17%)	PGF2-α[c]	
India	183	Endometritis (18%)	E. coli LPS[d] IU	84% CR[e] (3AIs)
		Luteal insufficiency (18%)	Progesterone injections	CR up by 10%
Indonesia	6/68	Anoestrus (Brahman)	GnRH	Ineffective
Myanmar	11	Anoestrus	Progesterone	5 (45%) showed oestrus
Pakistan	110	Endometritis	Antibiotics	103 (94%) cured
Philippines	65	Endometritis Anoestrus Ovarian cysts	Results awaited	Results awaited
Sri Lanka	9	Non detected oestrus in Buffalo	"Ovsynch"	CR 55%
Thailand	455	Repeat breeding Anoestrus	PGF2-α Vitamins ADE	CR up 10% 20% in heat

[a]GnRH = Gonadotrophin Releasing Hormone, [b]IU = Intrauterine, [c]PGF2-α = Postaglandin F2-α, [d]LPS = Lipopolysaccharide, [e]CR = Conception Rate

TABLE IV. IN-COUNTRY TRAINING CONDUCTED FOR FARMERS AND LIVESTOCK PERSONNEL (NO. OF COURSES / NO. OF PARTICIPANTS)

Country	Farmers	Veterinarians	AI Technicians	Scientists
Bangladesh	49/1739	1/22	4/37	0
China	3/35	2/34	0	1/6
India	17/597	14/104	0	7/45
Indonesia	5/68	3/28	3/29	3/30
Malaysia	4/12	0	2/10	0
Myanmar	17/262	6/55	6/40	1/1
Pakistan	20/682	1/5	5/108	0
Philippines	9/135	2/30	4/60	0
Sri Lanka	4/65	8/80	0	2/2
Thailand	2/76	0	2/60	0
Total	130/3639	37/264	26/344	14/57

In addition, to the results listed in Tables II to IV, the following notable achievements were obtained by specific countries:

- Thailand: a RIA kit has been developed and validated (but a problem exists with the binding of the monoclonal antibody to the progesterone tracer, and the cause needs to be determined and rectified);
- Indonesia: Production of progesterone tracer, polyclonal antibodies and standards is in progress;
- Bangladesh: Cattle health recording database has been developed;
- China: Solid phase sampling method (samples dried onto filter paper) has been developed for use in progesterone assays; progesterone measurement was used to diagnose reproductive problems in exotic (zoo) animals;
- India: Uterine defense modulation through E. coli lipoplysaccharide (LPS) or oyster glycogen administered intra-uterine has been developed for treatment of endometritis.

4. CONCLUSIONS

- The studies conducted under the project have established the following fertility parameters for small-holder cattle farms as the existing situation in the participating MSs: conception rates ranged from 27–73%; calving intervals ranged from 397–550 days.
- The main problems identified were: late age at first calving; low conception rates; repeat breeding and long calving intervals.
- The main reasons for the above problems were determined as: untrained AI technicians; poor heat detection; poor quality of semen; malnutrition; improper AI timing; and uterine infections.

- Evaluation of the effectiveness of AI using three strategic progesterone measurements on the day of AI and on days 10–12 and 21–24 later, followed by manual pregnancy diagnosis at 50-60 days after AI, showed that: incorrectly timed AIs ranged from 5.3–37.5%; AIs at ovulatory oestrus ranged from 50–83%; successful AIs were from 32–63%; and late embryo losses were 0–18.6%.

- The interventions undertaken resulted in improvements in conception rate ranging from 2.9–27 % in the different MSs.

- The training activities conducted in the different MSs included 130 events for farmers, 37 for veterinarians, 26 for AI technicians and 14 for scientists.

5. RECOMMENDATIONS

- The country reports should be edited and published as an IAEA TECDOC, which should also be placed on the RCA web site. Distribution and promotion of the results of this project to other MSs should result in considerable spill-over benefits to other locations where similar problems inhibit ruminant production.

- The project has developed and proven several technologies (listed above). All participating MSs should capitalize on these new possibilities by supporting wider extension of the practices and packages for the development of their respective livestock industries.

- The project activities are fully complimentary to national programmes for improving livestock nutrition and breeding in participating MSs. The excellent achievements made by participating groups in this project should be used for attracting additional funding from international sources enabling extension of the benefits to wider farming communities.

By the close of official activities, the project had already contributed notably towards increasing the productivity of livestock in the RCA countries, largely by increasing the production of milk and meat. This, in turn, enhanced the incomes of the participating farmers. In addition, due to the establishment of close coordination between this project, national projects and coordinated research programmes, several laboratories have received complementary assistance for upgrading their facilities and for consolidating their experimentation capabilities in the field. Due in part to this increase in the capacity and expertise of the laboratories, a new project was proposed for the 2005–2006 TC cycle. It has already been initiated and it focuses on improving livestock productivity by using indigenous resources whilst conserving the environment. The reproduction and breeding component of this new project addresses strategic use of crossbreeding while developing the adoption of programmes designed to improve the competitiveness of indigenous genetic resources.

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The authors wish to thank P. Ball and Amarjit Singh Nanda for their contributions to the manuscript. P. Bell and Amarjit Singh Nanda served as experts at the final meeting of the project and summarized the major results and accomplishments of the project.

USE OF MILK PROGESTERONE RADIOIMMUNOASSAY AND COMPUTER APPLICATIONS FOR COMMUNITY BASED REPRODUCTIVE HEALTH SERVICES IN SMALLHOLDER DAIRY FARMS OF BANGLADESH

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Abstract

USE OF MILK PROGESTERONE RADIOIMMUNOASSAY AND COMPUTER APPLICATIONS FOR COMMUNITY BASED REPRODUCTIVE HEALTH SERVICES IN SMALL HOLDER DAIRY FARMS OF BANGLADESH

We trained veterinarians, inseminators and farmers to increase their skills and compliance with project activities, used AIDA Asia (a computer application) to record and evaluate fertility of bulls and performance of inseminators, introduced measurement of progesterone (P4) in milk by radioimmunoassay (RIA) to detect artificial insemination (AI) done at incorrect time and non-pregnant cows, and adopted community-based veterinary services for the management of dairy cattle health and reproduction.

Training inseminators increased their skills in doing AI correctly and training veterinarians improved their confidence in the management of reproductive problems in cows. Training farmers increased their compliance to adopt the interventions that were introduced. Milk preserved with sodium azide maintained stable P4 concentrations for at least two weeks. Milk P4 concentration was intermediate (≥1 – <3 nmol/L) or high (≥3 nmol/L) on the day of AI (day 0) in 28% of inseminated cows, indicating AI was done at the incorrect time. About 16% of cows had low (>1 nmol/L) P4 concentration in milk on day 22–24 after AI, indicating non-pregnancy. More than 12% of cows that did not return to heat by 35–60 days after AI were non-pregnant upon rectal palpation. One-hundred and thirty-eight such cows were examined and 52% were anoestrous, 11% were repeat breeders, 14% had uterine infections and 17.4% had escaped oestrus detection. Various treatment regimes were tested for reproductive problems including anoestrus, repeat breeding and uterine infections, and those proven to be successful were adopted of wider application. Zebu bulls achieved 3.5% higher conception rate (50.8%; n = 1275) than did crossbred bulls (47.3%; n = 1256). Conception rates achieved by individual inseminators ranged between 45.5% and 54.1%.

In conclusion, milk progesterone RIA identified AIs done at the incorrect time and non-pregnant cows; on-farm veterinary services identified non-detected oestrous cows, which could be treated and inseminated; and AIDA Asia proved useful in evaluating fertility of bulls and skills of inseminators.

1. INTRODUCTION

The dairy industry in Bangladesh started growing remarkably from the beginning of 1990. The number of dairy farms increased from 2490 in the year 1990–91 to 29 600 by the year 1997–98 [1]. Milk production increased from 1.29 million metric tons in 1987–88 to 1.74 million metric tons in 2001. Due to increased production, milk powder imports have decreased from 55 000 metric tons in 1991–92 to 17 000 metric tons in 2001. However, per capita milk availability is quite low [2]. Assuming a daily per capita requirement of 250 mL, the dairy industry in Bangladesh must grow at an annual rate of 4.2–5.6% to meet the increased demand of an expected 1.6% population growth by 2010.

The reasons hampering further growth of the dairy industry in Bangladesh were identified to be, among others, feed shortage, widespread infectious and production diseases and inefficiencies leading to low productivity [3]. Our earlier studies identified the main constraints to be prolonged postpartum intervals to conception and low conception rate (CR), which were the results of inefficiencies in the management of nutrition, oestrus and artificial insemination (AI) services [4]. An economic opportunity survey showed that management improvements directed towards increasing milk production, increasing lactation length, decreasing age to first calving, decreasing calf mortality and decreasing calving interval could increase income by $329–807 per farm per year, depending on the location [5]. A participatory rural appraisal demonstrated that the main demand of farmers was for on-farm services that would address feeding, health and problems related to reproduction, and that the resulting increased income would enable farmers to pay for such services [6]. Priorities identified by the farmers were: training for themselves on the management of farms to obtain good profit margins and training for inseminators and veterinarians to make them capable of delivering effective services [6].

There is thus a need to change the traditionally hospital based emergency veterinary service to an on-farm production-oriented one. The benefits demonstrated by herd health services in developed dairy industries may be hard to implement in Bangladesh because dairy farms here contain very few cows. This means an alternative, on-farm service delivery system needs to be developed for small dairy farms. In Bangladesh, India and some other countries, smallholder dairy farmers' cooperatives were successful in developing milk markets and delivery of inputs [7]. Therefore it is likely that a community based, on-farm service for the management of nutrition, health and reproduction could be delivered through the farmers' cooperatives. We report here results of training veterinarians, inseminators and farmers to increase skills in their respective fields, application of a data recording and analysis system coupled with measurement of progesterone (P4) in milk by radioimmunoassay (RIA) to improve AI services and establishment of on-farm production-oriented, community based veterinary services to increase reproductive efficiency of cattle in selected areas of Bangladesh.

2. MATERIAL AND METHODS

2.1. Training

2.1.1. Veterinarians

Theory and practical training of one week duration on 'The Management of Dairy Cattle Health and Reproduction' was organized at Bangladesh Agricultural University, Mymensingh with the collaboration of the Central Cattle Breeding Station, Savar, Dhaka from 09 to 14 February 2002. Twenty-two veterinarians working with the project, from the

Department of Livestock Services, Bangladesh and Bangladesh Milk Producers' Cooperative Union Ltd., Dhaka, participated in the training. The training included lectures and demonstrations for 3 days and on-farm practicals for 3 days. About 60 cows were used in the practical sessions. The training focussed on examination of cows for reproductive status, pregnancy diagnosis by day 35–40 after AI, understanding infertility and conception failure, oestrus management and programmed reproduction, economic management of postpartum dairy cattle, udder health and the mismanagement of calves and replacement heifers.

2.1.2. Inseminators

Insemination skills of 37 inseminators participating in AI programmes supported by the government, farmers' cooperatives and Non-Governmental Organizations (NGOs) were evaluated during 11 December 2001 and 8 May 2002 in 4 batches. Individual inseminators were asked to thaw a straw of frozen semen. The thawing time adopted by the inseminators and the temperature of the water used were recorded. They were then asked to simulate an insemination by depositing gentian violet dye in the genital tracts removed from slaughtered cows. The genital tracts were dissected after simulated AI to determine the site of dye deposition. The inseminators were then trained for two days in proper AI procedures. A repeat evaluation on semen thawing and dye deposition was done after the training.

2.1.3. Farmers

Training camps of one-day duration on 'Farmers Training on Economic Dairy Farm Management' were held between 6 December 2001 and 30 June 2002. The numbers of farmers participating were 545 from Mymensingh, 400 from Khulna-Satkhira, 395 from Sirajgong-Pabna and 396 from Chittagong. The training was completed in 49 batches, with individual batches comprising 30–40 farmers.

The farmers were introduced to the project objectives, work plan and expected outcome. Different aspects of profitable dairying such as feeding management of cows, general and reproductive health management, udder health management, and the preparation and feeding of urea molasses mineral blocks were discussed. A farmer training manual with 53 illustrations was provided. The importance of keeping records on cows was discussed and a simple method employing a breeding calendar was introduced. Its routine use was fully explained.

Farmers were informed on proper feeding of dairy cows using roughage and concentrate. The recommendation for a cow yielding up to 5 kg milk/day was about 10–20 kg green forages, 4–5 kg straw and 250 g *Sesbania* leaves or *Ipil Ipil* (*Leucaena*) leaves along with 1 kg rice polish, 1 kg wheat bran, 1 kg sesame oil cake and 100 g iodinated salt. We recommended splitting the total feed in to three separate meals. In relation to health and reproduction management, the following information was delivered: (a) the primary sign of oestrus is standing to be mounted, but the most important secondary sign is discharge of clear mucous from a swollen vulva; (b) insemination between 12 and 18 hours after the onset of oestrus achieves highest CR; (c) non-pregnancy can be confirmed by determining milk progesterone concentration using RIA between days 21 and 24 after AI and the information can be delivered to the farmers by day 35 (before next oestrus); (d) calves should receive colostrum as early as possible and their umbilicus should be disinfected with gentian violet; (e) calves should be kept on a raised platform; (f) the barn should be disinfected regularly; (g) deworming of calves should begin at 3 months age and vaccination at 6 months of age; (h) udder and teats should be cleaned properly before milking by dry wiping and then the teats should be dipped in potassium permanganate; (i) teat dipping should be repeated after milking; (j) cows should be given enough feed immediately after milking so that they remain standing for at least 2 hours; and (k) farmers should use strip cup method to test for clinical mastitis and call the veterinarian if there are indications of infection.

Afterwards, the farmers were divided into several small groups. They discussed among themselves the technologies delivered through the training and other problems related to dairying. The session ended with a discussion of farmers' problems with the responsible principal investigator(s) and the training camp ended with a concluding session.

2.2. Progesterone concentrations in milk preserved at different temperatures

Milk was collected in screw-capped tubes containing a sodium azide tablet as preservative, on days 22–24 after insemination, from 10 local non-descript zebu cows, 10 zebu cows of Pabna-Sirajgonj areas of Bangladesh (popularly called Pabna Milking Cow, PMC) and 10 crossbred cows (zebu dams and Friesian sires). Milk samples from individual cows were transported and preserved according to any one of the four following procedures:

(i) Milk was preserved at 4°C immediately after collection, transported maintaining them at 4°C and preserved at 4°C in the laboratory until defatted.

(ii) Milk was preserved at 4°C immediately after collection, transported to the laboratory at ambient temperature and then preserved at 4°C until defatted.

(iii) Milk was collected and transported at ambient temperature and preserved at 4°C in the laboratory until defatted.

(iv) Milk was collected and transported at ambient temperature and preserved at room temperature in the laboratory until defatted.

Milk samples were defatted two weeks after they were collected by centrifugation at 1500 rpm for 15 minutes at 4°C and the skim milk was frozen at –80°C until progesterone measurement by RIA.

2.3. Non-pregnancy diagnosis

Milk samples at day 0 and day 22–24 after AI were collected from the farms registered with the project and stored in screw-capped tubes containing a sodium azide tablet. Inseminators collected the day 0 samples at the time of AI. Farmers collected the day 22–24 samples and gave them to the inseminators or veterinarians. The inseminators or veterinarians sent the samples to the laboratory. The RIA for progesterone was performed in the laboratory using a solid-phase system employing a monoclonal antibody and ^{125}I labelled progesterone tracer (FAO/IAEA 'self-coating' assay).

2.4. Reproductive health management

Farm visits were made and 'Fertility Control Camps' were held between April and July 2004. Farms with 5 or more breedable cows were visited by the veterinarians once a month. Cows and heifers bred 35 days earlier were examined per for pregnancy by rectal palpation. Cows bred on three consecutive occasions without conceiving were examined for reproductive problems. Cows that did not show oestrus by day 60 postpartum were examined to diagnose cyclic status. Heifers that were more than two years old but had not shown oestrus were examined for cyclicity. Farmers with fewer than 5 breedable cows were invited to bring their problem cows and heifers into fertility control camps organized in the community.

Anoestrous heifers with neither a palpable corpus luteum (CL) nor a follicle on the ovaries were either treated with 3 intramuscular injections of a combination of vitamins A, D3 and E (AD3E) or first dewormed and then treated with AD3E. Anoestrous heifers and cows without a palpable CL but with palpable follicles were treated with GnRH followed 12–14 days later by prostaglandin F2α (PGF2α) and were either inseminated on observed oestrus or two AIs were done without oestrus observation at 70 and 90 h after the PGF2α injection and an additional GnRH injection was given at 70 h. Cows and heifers with a palpable CL on the ovaries were treated with PGF2α and AI was done on observed oestrus. Cows and heifers claimed to be repeat breeding but with clear genital discharge and a history of prolonged oestrus (more than 24 h) were either inseminated twice or treated with intramuscular injection of GnRH at the time of AI. Cows and heifers with cloudy genital discharge were treated with an intrauterine infusion of 2.0 million IU of procaine penicillin immediately after oestrus followed by AI 8 h later and two additional intrauterine infusions of 2.0 million IU procaine penicillin each at 24 and 48 h intervals after the first infusion.

The data were recorded in forms designed for farm visits and entered in a computer application that was developed in Microsoft Access XP. A macro was designed to export data from the MS Access application to MS Excel as multiple farm entries. Simple averages, frequencies and percentages were calculated.

2.5. AIDA Asia for evaluating fertility of bulls and performance of inseminators

The Artificial Insemination Database Application for Asia (AIDA Asia) was developed by FAO/IAEA in Microsoft Access 2000® and Visual Basic for Applications® 6.0 [8]. The computer application contains 6 files to record, analyse and report data on farms, females inseminated, semen, oestrus characteristics, inseminators and pregnancy diagnosis.

Fourteen bulls comprising 7 zebus and 7 crossbreds (50% zebu and 50% Friesian) that were used in the national AI programme were selected to determine their fertility. Ejaculates of the bulls were processed, frozen and distributed to 28 AI technicians in 28 different places in Bangladesh. From each bull 644 doses (total of 9016 for the 14 bulls) were randomly distributed to 28 inseminators. The semen was used only for the first service. All inseminated cows were planned to be examined per rectum for pregnancy diagnosis. Data on 2531 AI records were entered in AIDA Asia. Data were exported to MS Excel for preparing graphical presentations.

3. RESULTS

3.1. Training

3.1.1. Veterinarians

The participants shared their field experiences and gained confidence in delivering on-farm cattle health and reproduction related services. All of them agreed to visit the registered farms of the project. A decision was made to develop a working protocol for uniform practices on the management of udder health, infertility and calf health. Farms with five or more lactating cows were planned to be visited monthly as a routine practice. Owners of farms with fewer than five lactating cows were invited to fertility camps organized once a month in the project communities.

3.1.2. Inseminators

At the pre-training evaluation, only 25% and 72% of inseminators (n = 36) used the correct time and water temperature, respectively, for thawing semen (Table I). After training, all inseminators thawed the semen straw as recommended for time and temperature. At the pre-training evaluation, only 57% of inseminators deposited gentian violet dye in the body of the uterus (Table II; Figure 1a–e). In 2 (5%) cases the dye did not pass into the genital tract, instead flowed back through the space between the barrel of the insemination gun and the sheath. At the post-training evaluation, all inseminators successfully deposited the dye in the body of the uterus.

TABLE I. SEMEN THAWING TIME AND TEMPERATURE USED BY INSEMINATORS AT PRE-TRAINING EVALUATION (N = 36)

Thawing time and water temperature	Pre-training evaluation	
	Number	%
Thawing time (sec)		
3–9	15	42
10–12[a]	9	25
13–32	12	33
Thawing temperature (°C)		
31–34	3	8
35–38[a]	26	72
39–46	7	20

[a]Correct values

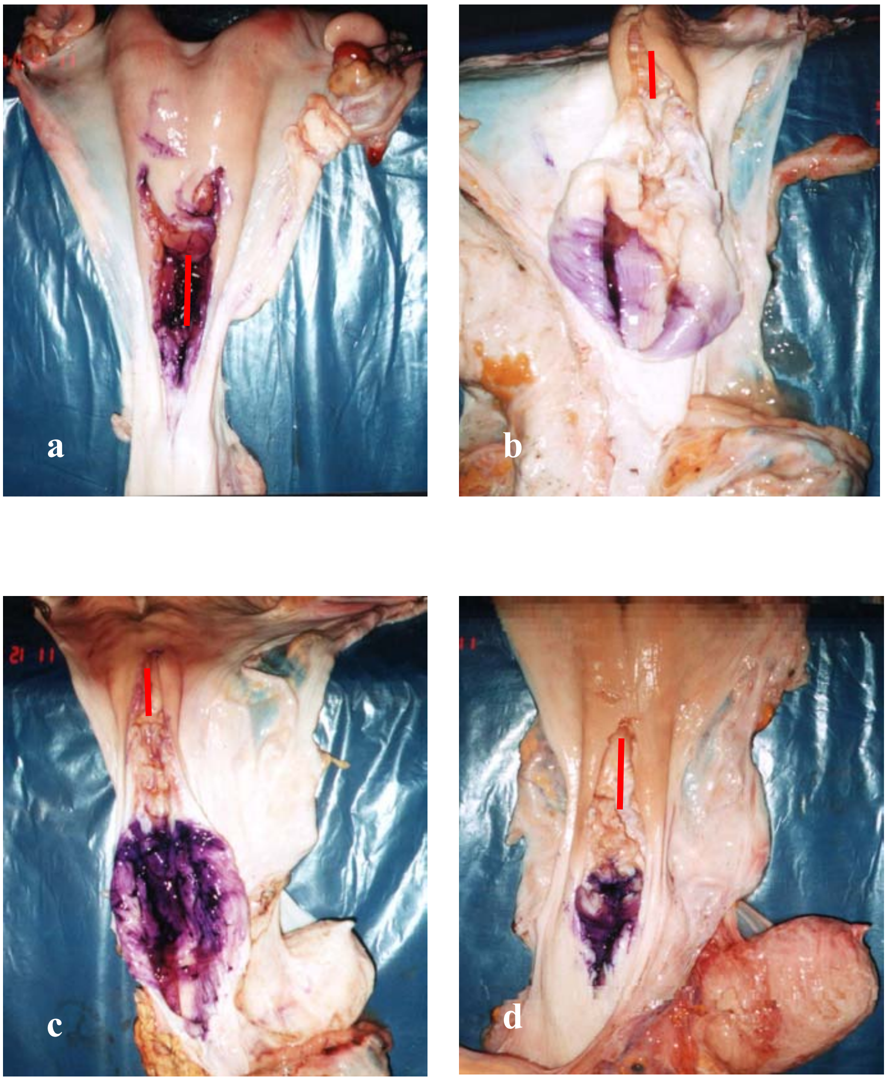
TABLE II. SITES OF GENTIAN VIOLET DYE DEPOSITION MADE BY INSEMINATORS IN EXCISED GENITAL TRACTS OF COWS AT PRE-TRAINING EVALUATION (N = 37)

Sites of dye deposition	Pre-training evaluation	
	Number	%
Anterior vagina	4	44
Between the rings of the cervix	7	19
Body of the uterus[a]	21	57
Horn of the uterus	3	8
Back flow[b]	2	5

[a]Correct location
[b]No dye found in the opened genital tract

3.1.3. Farmers

Farmers shared their knowledge on dairying during group discussion sessions. They agreed to accept milk progesterone RIA for non-pregnancy diagnosis and to follow recommendations on reproductive and udder health management. Many farmers shared their experience on feeding of Urea Molasses Multi-nutrient Blocks (UMMB) and reported increased production and reproduction capacity of cows. They also agreed to prepare a breeding calendar and requested follow-up training at yearly intervals.



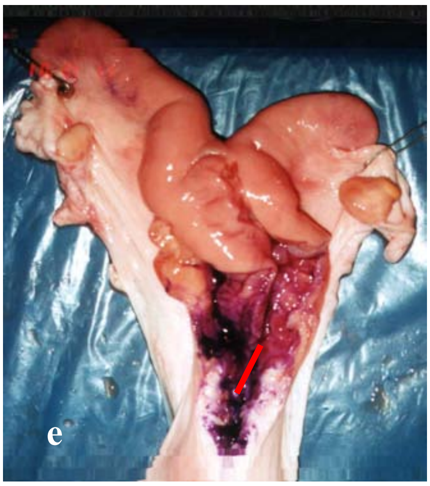


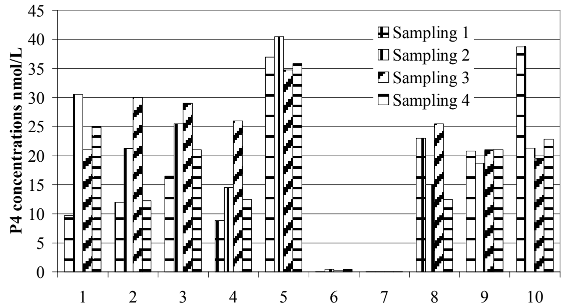
FIG. 1, a–e. Gentian violet deposited by the inseminators at (a) body of the uterus, (b) vaginal fold, (c) cervical opening, (d) between the first and second rings of the cervix and (e) into the left horn. Note - the red line indicates the position of the body of the uterus

3.2. Progesterone concentrations in milk stored at different temperature for two weeks

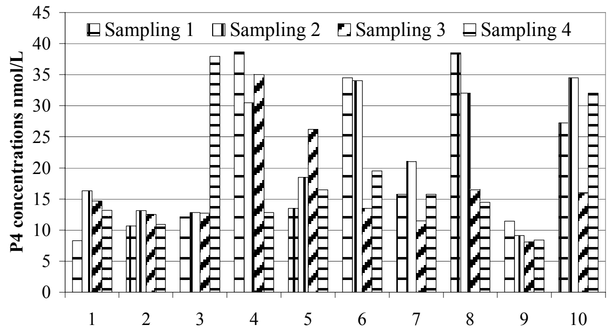
Twenty-eight of the 30 cows tested had high level of progesterone (≥3 nmol/L) in milk (Figure 2). This was consistent regardless of cow types and the four different sample managements. However, individual cow variations did exist with regard to the progesterone concentrations in milk.

3.3. Non-pregnancy diagnosis

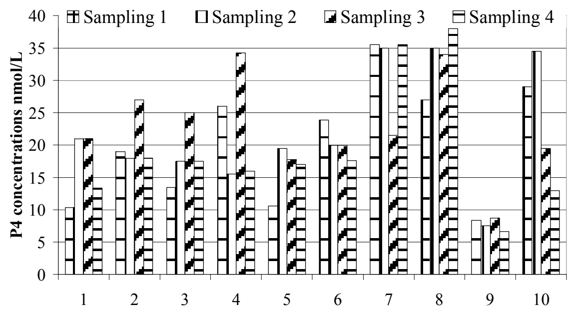
Progesterone concentrations in milk collected from cows at day 0 and day 22–24 are shown in Table III. Twenty-eight percent of inseminations were made at an incorrect time of the oestrous cycle. Sixteen percent of cows that did not show oestrus by day 22–24 after AI were not pregnant.



Crossbred cows



Local zebus in Mymensingh



Local zebus in Sirajgonj

FIG. 2. Progesterone concentrations determined by radioimmunoassay of defatted milk sampled four different ways from 3 types of cattle. Sampling 1 = preserved at 4°C immediately after collection and maintained at this temperature until defatted, Sampling 2 = Preserved at 4°C immediately after collection, transported to the laboratory at ambient temperature and then preserved at 4°C until defatted, Sampling 3 = collected and transported at ambient temperature and preserved at 4°C in the laboratory until defatted, Sampling 4 = collected and transported at ambient temperature and preserved at room temperature until defatted.

TABLE III. PROGESTERONE CONCENTRATION IN MILK COLLECTED ON THE DAY OF AI (DAY 0) AND AT DAY 22–24 AFTER AI

Day[a]	Total samples	No. (%) with low (<1 nmol/L) progesterone	No. (%) with intermediate (1–3 nmol/L) progesterone	No. (%) with high (>3 nmol/L) progesterone
Day 0	93	67 (72)	23 (25)	3 (3)
Day 22–24	90	14 (16)	8 (9)	68 (76)

[a]Day 0 and day 22–24 samples are not always from the same cow.

3.4. Reproductive Health Management

A total of 279 farms received reproductive health management services. One hundred eighty-two cows and heifers were examined for pregnancy and 159 (87.4%) were found to be pregnant. Twenty-three cows (13%) that did not return to oestrus by day 35–60 were not pregnant. AI services required per conception were 2.18.

Farmers presented 138 cows and heifers as having reproductive problems. The actual problems diagnosed in the different categories of complaints are shown in Table IV.

TABLE IV. DIFFERENCES IN COMPLAINTS OF FARMERS AND THE ACTUAL PROBLEMS DIAGNOSED

Complaint of Farmers	Number	Diagnosed problems	Number
Anoestrus	96	Anoestrus	79
		Unobserved and incorrectly reported oestrus	24
Repeat breeder	32	Cows with 3–8 services	15
		Cows treated as repeat breeders	21
Uterine infection	10	Uterine infection	14

TABLE V. OUTCOME OF DIFFERENT TREATMENTS USED FOR THE MANAGEMENT OF REPRODUCTIVE PROBLEMS IN CATTLE

Problem cows/heifers	Treatment used	No. recommended treatment	No. provided treatment	[a]No. with positive response	Treatment cost ($)
Anoestrous heifers	Vitamin AD_3E	23	23	11	2.0
	Anthelmintics + AD_3E	12	12	8	3.0
	[b]GnRH + PGF2α + 2AIs at 70 and 90 h + GnRH	21	16	10	10.0
Anoestrous cows	[c]GnRH + PGF2α + 2AIs at 70 and 90 h + GnRH	23	18	12	14.4
Repeat breeding	2 AIs at 12 h intervals	8	8	6	1.7
	2 AIs at 12 h interval + GnRH at first AI	13	13	10	9.3
Uterine infections	i/u procaine penicillin + AI + 2 procaine penicillin	14	14	11	5.4

Problem cows/heifers	Treatment used	No. recommended treatment	No. provided treatment	[a]No. with positive response	Treatment cost ($)
Cows claimed to be anoestrus but with CL	[c]PGF2α +AI + GnRH	24	12	11	10.0
Total		138	116	79 (68%)	

[a]Positive responses were: (a) in anoestrous cows and heifers — oestrus after treatment; (b) in repeat breeders and cows with uterine infection - non return to oestrus by day 30 or diagnosed pregnant by day 35–60
[b]Heifers were only inseminated in observed oestrus
[c]If a cow showed oestrus, only one insemination was done and the second GnRH was not injected

Treatments were prescribed for 138 cows and heifers; however, only 116 animals (84%) were actually provided treatment (Table V). Non-acceptance of the treatment by farmers was mainly in cases where the cost of drugs was high. The treatment costs varied from \$1.7 to \$14.4 depending on the problem identified and the drugs chosen. On average, 68% of treatments yielded positive responses.

The CRs resulting from inseminations done using semen from individual zebu and crossbred bulls and the CRs achieved by different inseminators are shown in Figure 3.

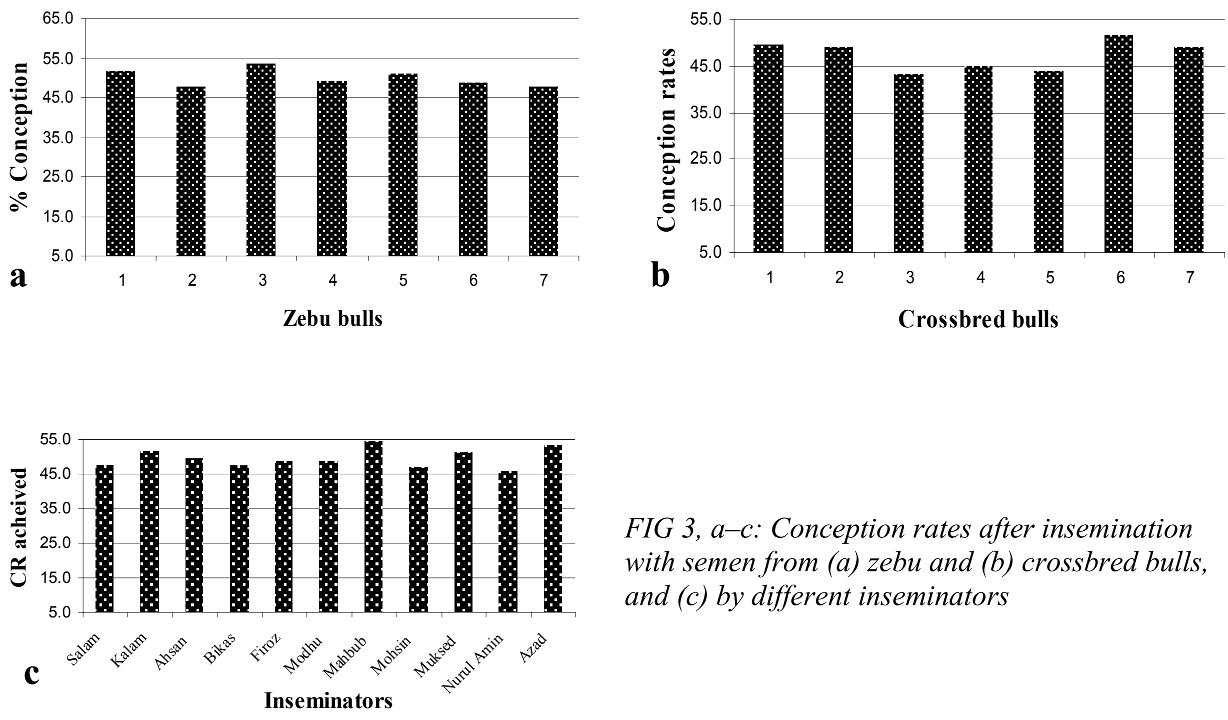


FIG 3, a–c: Conception rates after insemination with semen from (a) zebu and (b) crossbred bulls, and (c) by different inseminators

4. DISCUSSION

The main findings of the present study are that: (a) farmers miss oestrus in a high percentage of cows and heifers; (b) on-farm reproductive health management services identify the problem cows, which can be effectively treated; (c) training improves the performance of inseminators; (d) milk preserved with sodium azide maintains a stable concentration of progesterone up to two weeks; (e) milk progesterone measurement by RIA can be used to identify AIs made at incorrect time and to detect non-pregnant cows; and (f) AIDA Asia was useful in recording, analysing and reporting AI data.

Missing one oestrus extends the calving interval in cows and the age at first calving in heifers by 21 days. Recently we have estimated that economic losses of $43 and $11 occur when there is a delay of 21 days in age at first calving and calving interval, respectively [5]. Non-detected oestrus is the cumulative result of sub-oestrus and poor heat detection. Roger *et al.* [9] stated that farms with very good management might have 10–15% non-detected oestrus. The author regarded 15–20% non-detected oestrus as a slight problem and greater than 40% as a severe problem.

The present study does not have data on the total postpartum cows that are anoestrous by a defined day, which makes it difficult to calculate the percentage of non-detected oestrus. However, our earlier study, where ovarian cyclicity was evaluated by assaying progesterone in two milk samples collected every month at 10 day intervals, showed that 40% of postpartum cows were not detected in oestrus when they completed one or more ovarian cycles [4].

The majority of the farms we worked with had 2–6 breedable cows and 1–5 replacement heifers [5]. These cattle are tethered and stall-fed, a management practice that limited their interactions for the manifestation of primary oestrus signs. Another important issue is that AI technicians often state that a cow is in oestrous when she is not. In our earlier studies 30% of cows were stated to be in oestrus when they were not [4]. In the present study about 12% of 478 inseminated cows were not in oestrus [4]. Occurrence of such AIs were 9.6% (n = 3477) in China [10] and 14.8% (n = 204) in Myanmar [11]. Therefore, it is important that the farmers are aware of the secondary signs of oestrus. We organized 49 training camps of 30 to 40 farmers in each and discussed the opportunities for detecting more cows in oestrus if the cows are watched more frequently for secondary signs. Whether or not the training improved the skill of farmers in detecting oestrous cows could not be compared in this study. However, the training increased the awareness of farmers to report anoestrus cows to the veterinarians on farm visits.

The present pilot on-farm reproductive health management services benefited 279 farmers. Such a programme was requested by the farmers in PRA sessions [6]. A high number of problem cows were identified and treated with quite good success. Especially, it was worth informing the farmers that about 13% of cows that they had assumed to be pregnant were not and more than 17% of animals they had considered anoestrous were actually cycling. Further, more than 50% of cows that the farmers considered to be repeat breeders had 2 or fewer unsuccessful services, which can be considered as normal in Bangladesh. On-farm health management programmes are undoubtedly useful. However, the major challenge is to keep complete records in a manner that they could be retrieved, analysed and reported for evaluating the success of the services [12]. In the US, herd health services increased the income of farmers up to $85 per cow per year [13]. Similar services benefited farmers in New Zealand and the Netherlands with an increase of 1.2 kg milk per cow per day and 62 kg milk per cow per year, respectively [14, 15]. However, in a country like Bangladesh with very few cows per farm, health programmes developed for large herds may not be economically feasible. In this study, we used a community-based approach along with farmers' cooperatives using their society offices as venues for delivering the services in the form of camps. The service delivery went smoothly; however, collection of follow-up data and their recording and analysis would require more work. At present we are working to develop a computer application in Microsoft Access XP to store, analyse and report data on community based dairy cattle health, feeding, breeding and management of production.

A refresher training of two days duration significantly improved the skills of inseminators, as judged at the post-training evaluation. The insemination records revealed 2.9% higher CR compared to that of an earlier study (49.1% versus 46.2%) done from 1995 to 1999 [4]. Although the semen used was not from the same bulls, it was mostly from the same AI service provider. More important to mention here is that in the earlier study seven inseminators from the same population achieved CRs between 31.3% and 54% [4]. However, after training, 11 of the inseminators achieved CRs between 45.5% and 54.1%. This clearly indicated that training narrowed the differences in the performance of the inseminators. The need for training inseminators was prioritized as number one by the farmers in a PRA session [6].

In the present study, deposition of dye in the excised genital tracts of cows was used to determine the site of semen deposition by the inseminators. Other procedures widely used for this purpose include electrocauterisation, evaluating radiographic plates made during AI in living animals, linear array ultrasonography and biological dye deposition in live animals [16–19]. However, for many years, dye deposition in excised tracts has been in use because it is inexpensive, accurate and easy to apply [18]. Although some limitations do exist in the dye deposition technique, we succeeded in minimising the problems by using only a small amount of dye (0.25 mL).

The present study identified that about 15% of the cows with non-detected oestrus by day 22–24 after AI did not have a functional CL, clearly indicating that they were non-pregnant. In an earlier study, 100% of cows with low level of progesterone in milk by day 21–24 were found not to be pregnant at later per rectum examinations [4]. Similar investigations in China, Indonesia and Myanmar identified 25.8%, 22.6% and 11.8% of non-pregnant cows, respectively [10, 11, 20]. Milk progesterone estimation on day 22–24 after AI can be effectively used to identify non-pregnant cows once the participation of farmers and inseminators is ensured by appropriate motivation programmes. The procedure could be implemented through the proposed community based cattle health and reproduction services.

The fact that milk samples preserved with sodium azide can be stored up to two weeks at ambient temperature is important information for the application of the milk progesterone assay as a service with AI in farms of small holders. Such farms in Bangladesh are distributed over a wide distance from the AI Centres and Veterinary Hospitals. Samples often take a week to reach the laboratory through AI service centres. Alternatively, a cow-side milk progesterone assay would be more useful for the application of interventions on cows that are identified as non-pregnant by day 21 after AI.

The AIDA Asia database was found to be effective to evaluate the fertility of bulls and the performance of inseminators in the national AI programme of Bangladesh. In an earlier study we had successfully used AIDA as a research tool to evaluate the quality of AI services [4] and the present pilot study confirms its feasibility to be used also by the AI service providers. The 14 bulls we used had CRs varying from 44 to 54%. The CRs reported from China, Indonesia, Myanmar and Sri Lanka were 44%, 26%, 61% and 31–60% respectively [10, 11, 20, 21].

In conclusion, training improved the skills of inseminators and ensured the compliance of farmers and veterinarians with interventions prescribed under the project. A high percentage of cows and heifers were not detected in oestrus, and could be identified and treated through the application of on-farm reproductive health management programmes. Milk progesterone RIA was effective in identifying the non-pregnant cows and cows inseminated at incorrect time. AIDA Asia proved useful in evaluating the performance of AI services. Further work on developing a computer application to record and analyse reproductive health management data is essential to operate community based cattle health management programmes. A cow-side progesterone assay would be more useful than RIA for implementing effective and quick interventions on the cows that are found to be non-pregnant on day 21 after AI.

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ESTABLISHMENT OF SELF-COATING RADIOIMMUNOASSAY FOR PROGESTERONE COMBINED WITH SOLID PHASE SAMPLING TECHNIQUE FOR MILK AND ITS APPLICATION

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Abstract

ESTABLISHMENT OF SELF-COATING RADIOIMMUNOASSAY FOR PROGESTERONE COMBINED WITH SOLID PHASE SAMPLING TECHNIQUE FOR MILK AND ITS APPLICATION

The objective of this project was to improve dairy cow production in the Zhejiang Province of China. A field survey and sampling of milk for measurement of progesterone were done in 6 dairy farms. A solid phase sampling technique for milk using filter paper was established. Milk was stripped from the teats of the cow directly on to a piece of filter paper and dried. Dried milk samples were extracted with 90% ethanol. The average extraction recovery, verified by ^{125}I-progesterone, was 94.09 ± 3%. Samples of conventional liquid phase skim milk and extracted solid phase milk were assayed using a 'self-coating' radioimmunoassay (Sc-RIA) according to the protocol provided by the IAEA. The correlation between progesterone values in the two types of samples was 0.9682. The overall accuracy of early pregnancy diagnosis based on progesterone concentration in solid phase samples collected 23 days after insemination was 85.2% in pregnant cows and 96.8% in non-pregnant cows. On-farm interventions resulted in increased conception rates of 60–70% within 60–90 days after parturition. Measurement of progesterone in milk of cows was used for the diagnosis of reproductive disorders and appropriate treatments were provided. The assay method is also suitable for the measurement of progesterone in faeces in rare wild animals to study their reproductive physiology.

1. INTRODUCTION

The productivity of dairy cows in the People's Republic of China is low. In 1999, 4.42 million cows produced only 7.17 million tonnes of milk [1]. Of the total 30 000 cows in Zhejiang province, about 50% are raised in household farms and the rest in state-owned farms, yielding 250 000 tonnes of milk. Almost all cows are bred by artificial insemination (AI) under programmes run by governmental bodies, commercial companies and civil organizations. Various animal breeding centres and frozen semen banks supported by the Government and the Dairy Cow Farmer Association play an important role in dairy development. The AI work is done by the professional staff of the Animal Husbandry and Veterinary Stations (AHVS) at the levels of province, county, towns, villages and big dairy farms. In the dairy cow raising areas, each town or village has an AHVS, which supplies frozen semen to its professional staff to carry out AI services.

Long inter-calving intervals, which occur due to low conception rate (CR), long sexual inactivity after parturition and poor expression of oestrus, adversely affect the productivity of dairy cows. In state-owned livestock farms, a system for recording, follow-up and evaluation of AI activities is practiced, but it is not so in small private farms.

Radioimmunoassay (RIA) [2] has been used in China since the 1980s [3]. Equipment, staff and training in milk progesterone RIA are available in research institutes, which can help through: (a) diagnosis of early pregnancy after AI; (b) detection of onset of oestrous cycles after parturition; (c) ovarian dysfunction; and (d) detection of silent oestrus.

In order to improve dairy cow production in the Zhejiang Province of China, a project was undertaken by Zhejiang University with the cooperation of the Animal Husbandry and Veterinary Service Stations of Hangzhou City and JinHua City, and the Bureau of Animal Husbandry, Department of Agriculture, Zhejiang Province. The specific objectives were to establish and apply routine services to farmers, using the progesterone RIA as a diagnostic tool, to improve dairy cattle reproduction.

2. MATERIALS AND METHODS

Initially, the following activities were conducted: (a) establishment of a ^{125}I-tracer lab and training of young scientists in the technique of preparing tracer; (b) establishment of the methodology of milk progesterone RIA for dairy cows; (c) training of farmers and AI technicians with on-farm instructions; and (d) establishment of an RIA service centre in an area with concentrated dairy farming.

Subsequently, a field survey was carried out in 6 farms, each with 90–500 breeding cows, to determine current status of AI and to identify constraints. The farms were: Hangzhou Jinjiang Dairy Farm, Linan Zhengxing Dairy Farm, Zhejiang Nanhu Farm, Jinhua Duohu Dairy Farm, Jinhuan Hetang Dairy Farm, Dairy Farm of Jinhua Dairy Company and Experimental Farm of Animal Science College, Zhejiang University. The farms had nine AI technicians, with ages ranging from 22–51 years and experience of 1.5–23 years in AI. Four of them were veterinarians and the others had received training as veterinary assistants for 1 or 2 years. Three types of frozen semen were used *viz.* straw, pellet and ampoule, containing 15–40 million live sperms. Usually warm water at 34–36°C was used to thaw semen. Sperm motility in post-thaw semen ranged between 50–70%.

For skim milk samples, 10 mL of whole milk was collected in a tube containing one tablet of sodium azide as a preservative. Samples were centrifuged at 2000 g for 15 min within 2–3 days and the skim milk was separated and stored at –20°C until assayed for progesterone by the Self-Coating RIA (Sc-RIA) method using the protocol provided by the IAEA [4]. The counting efficiency was about 76% and the intra-assay coefficient of variation ranged from 0.13 to 7.58.

For solid phase milk samples, a piece of mid-speed qualitative filter paper (1.5 × 5.0 cm) was laid on a slide and a rubber band was used to fix it at one end. Milk was stripped directly from the teats of the cow on to the filter paper. The excess milk on the slide was poured away and the soaked filter paper was dried at 40–70°C under sunlight or a blower [5].

Progesterone was extracted from the filter paper using a modified method of Wasser *et al.* [6, 7]. The filter paper containing the dried milk sample was dipped for 30 min each in 10, 5 and 5 mL of 90% ethanol at 50–70°C. The extracts were pooled, condensed to 1 mL in an evaporator and assayed for progesterone by the Self-Coating RIA (Sc-RIA) method [4]. The extraction recovery from solid phase milk samples was determined using ^{3}H-progesterone and ^{125}I-progesterone and the average values were 95.21 ± 0.55 and 94.09 ± 3.03, respectively.

Milk samples collected using the two methods from the same cows were assayed to check the correlation between progesterone values. In order to establish the normal profile of progesterone changes as measured by Sc-RIA in cycling dairy cows, milk samples were collected by both methods from healthy non-pregnant cows every other day for 46 days.

Based on the limitations identified in the field survey, training activities were implemented to improve the knowledge and skills of AI technicians, veterinarians and farmers in Zhejiang Province. Training for technical staff included proper AI technique, recording and interpretation of data, and the diagnosis and treatment of reproductive disorders. On-farm training and instructions for farmers included improved management and feeding practices, including heat detection and keeping of records. For cows with reproductive problems, progesterone profiles and the previous history were used to arrive at a diagnosis. Appropriate treatment and/or advice on managing the problem were provided and the outcome was monitored.

3. RESULTS

3.1. Validation of sampling methods

Progesterone concentration in milk samples collected using the two methods (skim milk and solid phase filter paper) are given in Figure 1. They showed a high correlation of 0.9754.

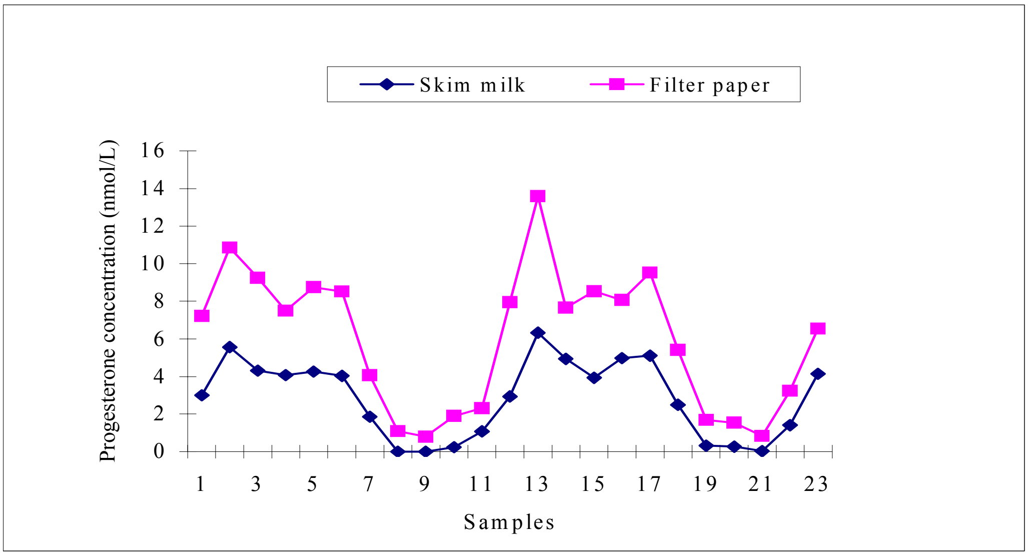

FIG. 1. Progesterone concentration in skim milk and in solid phase (filter paper) samples, which were collected every other day.

An example of the normal profile of progesterone changes as measured by Sc-RIA in skim milk of cycling dairy cows is shown in Figure 2.

3.2. Interpretation of results from solid phase (filter paper) sampling

Progesterone concentration in solid phase samples was less than 1.5 nmol/L on day 0 (day of AI) and above 6 nmol/L on days 10 and 23 after AI. Accordingly, analysis of solid phase samples collected on day 23 after AI from 847 cows gave an overall diagnostic accuracy of 96.8% in non-pregnant cows and 85.2% in pregnant cows. Interpretation of the reproductive status of cows based on progesterone concentration in three samples taken on days 0, 10 and 23 are given in Table I.

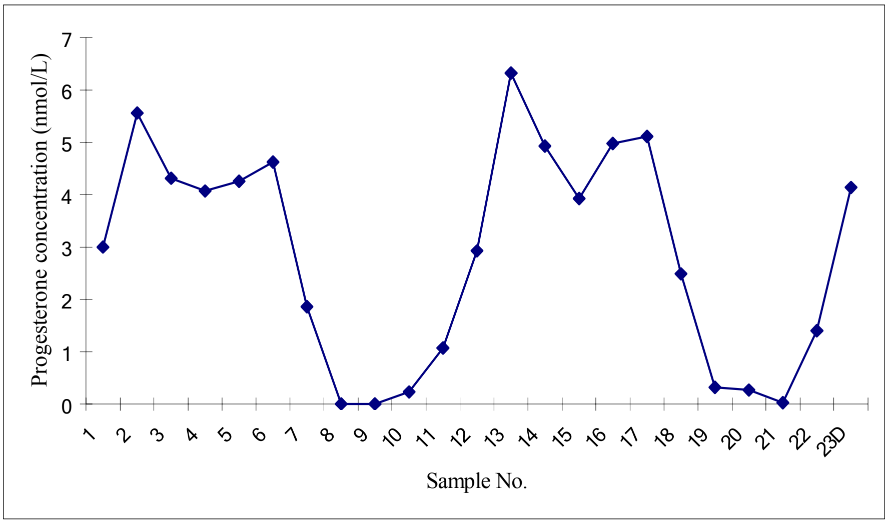

FIG. 2. Normal profile of changes in progesterone, measured using Sc-RIA, in skim milk of a cyclic dairy cow (samples were collected every other day)

TABLE I. INTERPRETATION OF REPRODUCTIVE STATUS OF COWS BASED ON PROGESTERONE CONCENTRATION IN THREE SAMPLES AND CLINICAL PREGNANCY DIAGNOSIS

Progesterone concentration[a]			**Clinical pregnancy diagnosis**	**Interpretation of reproductive status**	**No. (%)**
Day 0	Day 10	Day 23			
Low	High	High	Positive	Pregnant	561 (66.2)
Low	High	High	Negative	Late embryonic death or persistent CL	33 (3.8)
Low	High	Low	Negative	Non-fertilization or early embryonic death	98 (11.6)
Low	Low	Low	Negative	Acyclic	26 (3.0)
Low	*(High)	High	Positive	Pregnant	74 (8.7)
Low	High	*(Low)	Negative	Non-fertilization or early embryonic death	8 (0.9)
*(Low)	High	High	Positive	Pregnant	21 (2.5)
*(Low)	High	Low	Negative	Non-fertilization or early embryonic death	11 (1.3)
High	High	High	Positive	AI During pregnancy	3 (0.4)
High	Low	High	Negative	AI During luteal phase or irregular oestrus	12 (1.4)
Total					847

[a]When diagnosis was carried out using solid phase (filter paper) samples, Low and High indicate that the progesterone concentration was below 1.5 nmol/L and above 6 nmol/L, respectively. *(Low) and *(High) indicate that the progesterone concentration was intermediate (2.5–6 nmol/L)

3.3. Calving to first service interval

For 1400 cows from three of the six the farms used in the study during 2001 and 2002, the average intervals from calving to first service and to conception were 110.6 ± 43.0 and 119.4 ± 52.7 days, respectively. From the field survey data on all six farms, the first service conception rate (FSCR) and the overall conception rate (OCR) were 62.4% and 73.7%, respectively.

3.4. Effect of AI technician, environment and other factors on conception rate

The OCR achieved by the 9 technicians ranged from 43.5% to 82.7%, with the more experienced technicians achieving higher rates. The CR in cows served during the forenoon and evening was 61.8%, compared with 64.3% for those served in the afternoon. There was no difference in fertility when AI was performed 6, 8 or 12 hours after the first detection of oestrus. The OCR in spring, autumn and winter was higher (75%) than that in summer (55%).

3.5. Progesterone profile in cows with reproductive disorders

Progesterone profiles in two anoestrus cows are shown in Figures 3 and 4. Progesterone remained below 1.0 nmol/L in skim milk and below 2.5 nmol/L in solid phase samples. In the absence of oestrous signs, it was inferred that the ovaries were inactive. However, clinical examination revealed that the cow whose progesterone profile is shown in Figure 3 had a corpus luteum, but the low progesterone concentration indicated that the CL was non-functional.

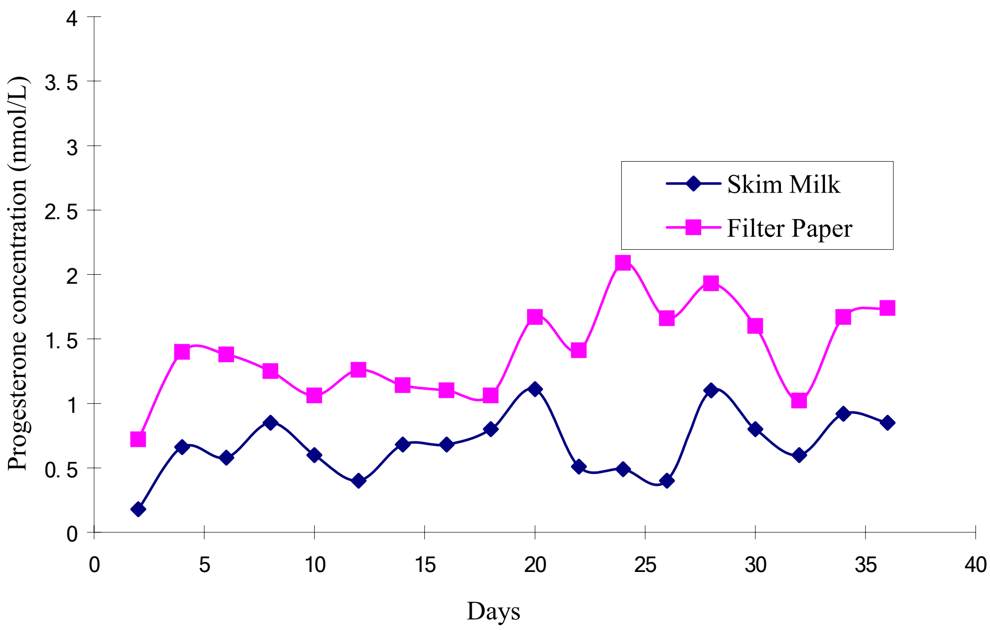

FIG. 3. Progesterone profile using skim milk and solid phase (filter paper) samples in a cow with a palpable Corpus Luteum (CL). The low progesterone values indicated that the CL was non-functional.

The cow whose progesterone profile is shown in Figure 4 had inactive ovaries. The correlation between the progesterone values from the two sampling methods was low due to the low progesterone concentration, leading to greater assay variability, relative to the mean.

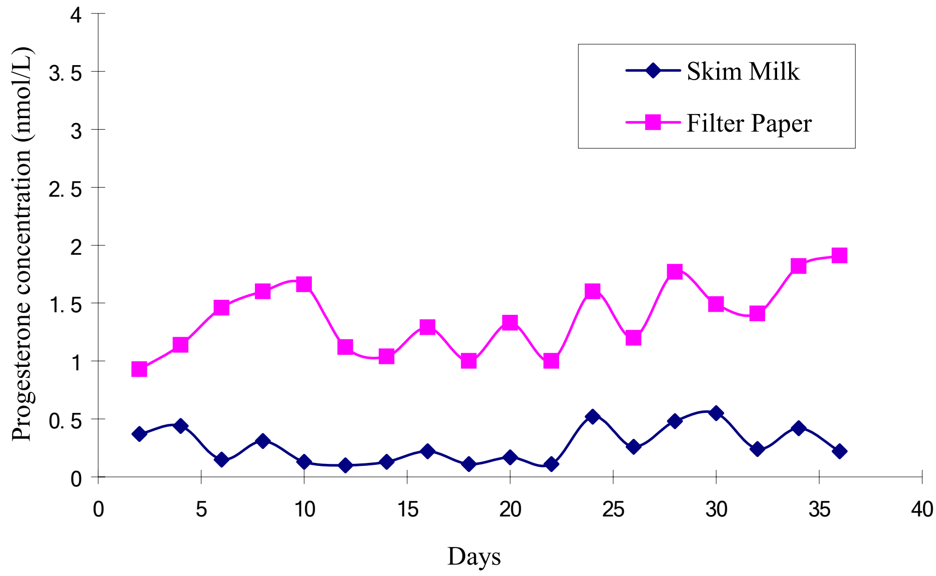

FIG. 4. Progesterone profile using skim milk and solid phase (filter paper) samples in a cow with inactive ovaries.

The training activities conducted for AI technicians, veterinarians and farmers resulted in an overall improvement of the OCR, reaching from 60% to 90% on individual pilot farms, within 60–90 days post-partum. The therapeutic treatments provided to problem breeders that were identified through progesterone measurement resulted in an overall recovery rate of around 80%.

4. DISCUSSION

According to a recent study in China, the low efficiency of AI was found to be due to poor heat detection and wrong timing of AI, the latter being as high as 18–20% [1, 8]. The present project included activities such as training of farmers and AI technicians, a field survey for determining the current status of AI and identifying constraints, and on-site instructions to livestock farmers.

Improvements in AI services and farm management were achieved through the training activities and on-farm instructions. Interventions were undertaken in the form of improved heat detection, correct timing of AI, keeping of records and provision of treatments for problem breeders. These activities lead to increased OCR of 60–70% within 60–90 days after parturition at the farms where the study was done. Accordingly, the authors strongly recommend that education, training and extension activities to improve the knowledge and skills of veterinarians, AI technicians, extension staff and farmers should be strengthened in China.

Low CR in Zhejiang Province has been shown to be due to two main factors. Firstly, disorders of the reproductive system, especially endometritis, have a high occurrence of around 17% [9, 10] and often cause early embryonic death and abortion leading to long calving intervals. Secondly, CR is lower in summer than during other seasons. Because Zhejiang province is in the south of China, the average temperature in summer ranges from 32 to 37°C, and heat signs cannot be observed during the day but can only be seen at night.

Therefore, more attention should be paid to studies on preventing reproductive disorders and improving heat detection in this Province.

A functioning RIA laboratory using the Sc-RIA method was set up for the assay of progesterone in skim milk and solid phase (filter paper) samples. The results indicate that the performance of the Sc-RIA method can match that of the more expensive commercial kits that use antibody pre-coated tubes. Therefore the Sc-RIA has good potential for application as a routine method in early diagnosis of non-pregnancy and reproductive disorders on cattle farms.

Since preservation of biodiversity is a national and global issue, the Sc-RIA facility established under this project was used in a preliminary study to determine progesterone concentration in the faeces of elephants and giraffes. The results indicate that faecal progesterone levels in these species also are indicative of their reproductive status. Thus the Sc-RIA laboratory can be used in the study of reproductive physiology and the diagnosis of pregnancy in wild and zoo animals.

ACKNOWLEGEMENTS

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APPLICATION OF A COMPUTER DATABASE AND PROGESTERONE RADIOIMMUNOASSAY FOR THE ASSESSMENT OF FACTORS AFFECTING CONCEPTION RATE IN CROSSBRED COWS FOLLOWING ARTIFICIAL INSEMINATION UNDER FIELD CONDITIONS

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Abstract

APPLICATION OF A COMPUTER DATABASE AND PROGESTERONE RADIOIMMUNOASSAY FOR THE ASSESSMENT OF FACTORS AFFECTING CONCEPTION RATE IN CROSSBRED COWS FOLLOWING ARTIFICIAL INSEMINATION UNDER FIELD CONDITIONS

The study was conducted to identify various factors affecting the success of artificial insemination (AI) and to improve reproductive efficiency through better management, AI services and application of progesterone radioimmunoassay (RIA). Data pertaining to 568 inseminations in cattle were collected according to the protocol of the Artificial Insemination Database Application (AIDA). Of these, 149 cows were subjected to clinico-gynaecological examination and milk samples were collected for progesterone assay to determine possible causes of infertility and the success of various interventions. Highest conception rates were obtained when the cows were inseminated during standing oestrus (51.6%), 18–23 h after the onset of oestrus (48.8%), by inseminators with a professional degree (48.7%) and using the best quality semen (51%). The main causes of repeat breeding identified during clinical and laboratory studies were: (a) inappropriate timing of AI in 48% of cows (milk progesterone concentration >1.0 nmol/L at AI); (b) subnormal luteal phase in 15% (plasma progesterone concentration < 4.77 nmol/L); (c) prolonged oestrus (>36 h) in 15%; and (d) sub-clinical microbial endometritis in 18%. Use of poor quality semen also contributed to repeat breeding.

1. INTRODUCTION

Artificial insemination (AI) has remained the main tool for dissemination of outstanding germplasm, control of venereal diseases and cost-effective dairy farming. India started a programme of crossbreeding of low producing zebu cattle with exotic germplasm through AI with an aim to enhance milk production. Although this strategy improved the milk production to some extent, the conception rate (CR) following AI was very low, around 30% [1]. The precise cause of this failure of AI, however, is unknown. The resulting decrease in rates of reproduction had direct economic implications on the Indian dairy industry and warrants identification of the aetiological factors involved and formulation of appropriate interventions.

Monitoring the success of AI through conventional methods, *viz.* rectal palpation of genitalia and non-return rate, has limited value. Measuring the progesterone profiles of cows by radioimmunoassay (RIA) has been of help to assess the suitability of animals for AI, monitor pregnancy and diagnose factors limiting reproductive efficiency [2]. Such studies on crossbred cows in India are obscure. There is a need to undertake a comprehensive assessment of fertility and to identify various factors affecting the success of AI. The present study was therefore undertaken with the following objectives: (a) assess the current status of AI services in India; (b) analyse problems in reproductive management and the delivery of AI services; and (c) study the efficacy of milk progesterone RIA for diagnosis of early non-pregnancy following AI and for diagnosis of factors affecting the success of AI.

2. MATERIALS AND METHODS

2.1. Field survey

A field survey was conducted in Punjab state using a questionnaire prepared in accordance with the protocol for the Artificial Insemination Database Application (AIDA) computer software program provided by the FAO/IAEA. AIDA is designed to store and analyse data on various aspects of AI. The required information was collected for 568 inseminations done by 34 AI technicians in 446 crossbred cows belonging to 89 farmers. The following data was recorded on each inseminated cow, entered in AIDA and analysed statistically:

2.1.1. Factors related to the cow

Quality of oestrus (standing oestrus or other signs); vulval swelling at oestrus (marked, slight or no swelling); quality of cervico-vaginal mucus (clear, turbid or no discharge); type of the preceding calving (normal or assisted); and uterine tone (marked or slight).

2.1.2. Factors related to farm management

Method of heat detection (visual or teaser bull); time interval from the first sign of oestrus to AI (hours); and housing system (loose barn or tie stall).

2.1.3. Factors related to semen

Quality of the semen (good or unknown) and method of thawing semen (in warm water, cold water or air).

2.1.4. Factors related to AI technique and the technician

Passage of the AI gun (easy or difficult) and education level of the AI technician (professional degree, diploma or progressive dairy farmer).

The main calculations done following AIDA analysis were the effects of these factors on first service conception rate (FSCR), overall conception rate (OCR) and the number of services per conception.

2.2. Monitoring the success of AI through progesterone analysis

Milk samples (10 mL) were collected from 149 cows into glass vials containing 100 mg of sodium azide on day of AI (day 0), and 10–12 and 20–23 days after AI. Samples were centrifuged at 3000 rpm for 15 min and refrigerated for 10 min, then the skimmed milk was transferred to glass vials and stored at –20°C until assayed for progesterone by RIA. Progesterone was measured using RIA kits (Diagnostic Products Corporation, USA) containing progesterone antibody pre-coated tubes and pre-diluted ^{125}I progesterone tracer, according to the protocol provided by the IAEA (Progesterone RIA ‘Pre-coated Tube’ Method, Assay Protocol Version 3.1, 1996).

The sensitivity of the assay was 0.10 nmol/L. The mean intra- and inter-assay coefficients of variation were 6.7% and 10.1%, respectively. Data on history, clinical observations and progesterone profiles were stored and analysed using AIDA.

2.3. Role of luteal inadequacy in repeat breeder cattle

Twenty crossbred cattle that were repeat breeders (i.e. failed to conceive after three normal inseminations) and six normally cycling cattle were selected from villages in Punjab. The body condition score of these animals varied from 2.5–4.0 [3]. Starting from the day of heat (day 0), the cows were subjected to clinical examination, per rectal palpation of genitalia and blood sampling *via* jugular venipuncture on days 0, 6, 7, 10, 12, 14 and 18. Blood plasma was separated by centrifugation and stored at –20°C until assayed for progesterone by RIA using kits (Diagnostic Products Corporation, USA) as described above.

2.4. Relationship between repeat breeding and prolonged oestrus in cattle

Twenty-two crossbred cattle that were repeat breeders (11 heifers and 11 cows, the latter in second to fourth parity) with a history of prolonged oestrus duration of up to 4 days (Group I) were selected for this study. They had clear cervico-vaginal mucous discharge. Six normally cycling cows with normal oestrus duration of up to 24 h were kept as controls (Group II). The genitalia of each animal were examined by rectal palpation and blood samples were collected at 3–5 day intervals for 1–2 consecutive oestrous cycles.

Plasma progesterone was measured by RIA as described above: profiles were plotted for each animal and correlated with history, clinical signs and findings from rectal palpation. Progesterone concentrations exceeding 1.59 nmol/L at the time of standing oestrus were defined as suprabasal concentration. Animals with progesterone concentration exceeding 4.77 nmol/L on the day of oestrus were excluded from the study. The approximate day of ovulation was deduced on the basis of history and the presence of a corpus luteum (CL) on rectal palpation.

2.5. Confirmation of pregnancy and statistical analysis

Pregnancy was confirmed on the basis of non-return to oestrus and by rectal palpation 2–3 months after AI. The data were analysed according to Student's t-test using differences between two means [4].

3. RESULTS

3.1. Identification of factors affecting the success of AI

Of the 568 AIs done on 446 cows, 218 resulted in conceptions, leading to an OCR of 38.4%. Of the 214 cows inseminated for the first time, 63 conceived, resulting in a FSCR of 29.4%. The findings according to the different factors related to the cow, bull and management are given in Table I.

3.1.1. Factors related to the cow

Of the 568 oestrus periods, 182 (32%) were classified as 'standing oestrus' at the time of AI and cows inseminated during this state had significantly higher ($P < 0.05$) FSCR and OCR compared to others that had exhibited only subsidiary heat signs like bellowing, restlessness, mounting behaviour and vaginal mucus discharge.

TABLE I. CONCEPTION RATE (CR) AFTER FIRST AND ALL SERVICES IN COWS IN RELATION TO DIFFERENT FACTORS

Factors or variables			First service			All services			Services per conception
			No. services	No. conceptions	CR (%)	No. services	No. conceptions	CR (%)	
Related to the cow	Heat signs	Standing heat	60	26	43.3	182	94	51.6	1.9
		Other heat signs	154	37	24	386	129	32	3.1
	Vulval swelling	Marked	86	36	41.9	256	118	46.1	2.2
		Slight	106	23	21.7	281	93	33.1	3.0
		None	22	4	18.2	31	7	22.6	4.4
	Vaginal discharge	Clear	203	63	31	547	217	39.7	2.5
		Turbid	7	0	0	16	1	6.25	3
		None	2	0	0	5	0	0	–
	Calving	Normal	155	51	32.9	410	161	39.3	2.5
		Abnormal	19	3	15.8	75	27	36	2.8
	Uterine tone	Marked	152	49	32.2	442	190	43	2.3
		Slight	62	14	22.6	126	28	22.3	4.5
Related to farm management	Heat detection	Teaser	117	32	27.4	297	111	37.4	2.7
		Visual	97	31	32	271	107	39.5	2.5
	Onset of heat to AI (hours)	<12	24	3	12.5	44	7	15.9	6.3
		12–17	106	34	32.1	270	104	38.5	2.6
		18–23	45	17	37.8	129	63	48.8	2.0
		24–29	27	8	29.6	89	32	36	2.8
		30–35	9	1	11.1	22	7	31.8	3.1
		36–42	1	0	0	5	3	60	1.7
		>43	2	0	0	7	2	33.3	3.5

TABLE I. (CONTINUED). CONCEPTION RATE (CR) AFTER FIRST AND ALL SERVICES IN COWS IN RELATION TO DIFFERENT FACTORS

Factors or variables			First service			All services			Services per conception
			No. services	No. sonceptions	CR (%)	No. services	No. conceptions	CR (%)	
Related to farm management (Contd.)	Housing system	Loose barn	122	33	27	319	112	35.1	2.8
		Tie stall	92	30	32.6	249	106	42.6	2.3
Related to semen	Semen quality	Good	30	11	36.7	75	38	50.7	2
		Unknown	184	57	29	493	193	37.1	2.7
	Method of thawing	Water at 37°C	162	53	32.7	431	188	43.6	2.3
		Cold water	45	6	13.3	120	22	18.3	5.5
		Air	7	4	57.1	17	8	47.1	2.1
Related to AI technique and the technician	Passage of AI gun	Easy	193	62	32.1	522	210	40.2	2.5
		Difficult	21	1	4.8	46	8	17.4	5.8
	Education level	Professional degree	106	38	35.8	306	149	48.7	2.1
		Diploma	39	8	20.5	103	21	20.4	4.9
		School certificate	69	17	24.6	159	48	30.2	3.3

The FSCR and OCR were significantly higher (P <0.05) in cows with marked vulval swelling at the time of AI (41.9% and 46.1%, respectively) when compared with those of cows with slight (21.7% and 33.1%, respectively) or no vulval swelling (18.2% and 22.6%, respectively). The FSCR and OCR in cows with clear mucus discharge were 31.0% and 39.7%, respectively. None of the cows with no mucus discharge or a turbid one conceived to a first service. Only one out of 16 AIs done in cows with a turbid mucus discharge was fertile.

The type of calving at the previous parturition had a marked effect on FSCR, being higher in cows with a normal calving (32.9%) compared to that in cows experiencing an abnormal calving (15.8%). Cows with marked uterine tone at AI had significantly (p <0.05) higher FSCR (32.2%) and OCR (43.0%) compared to those with slight uterine tone (22.6% and 22.3 %, respectively).

3.1.2. Factors related to farm management

The FSCR and OCR were similar in cows that were detected in heat either by visual observations or by a teaser animal. When the interval between the first sign of heat observed by a farmer and the performance of AI was divided into periods of 6 h each, the OCR was highest (48.8 %) in the cows inseminated at 18–23 h after the first observation. The type of housing appeared to have no significant effect on CR.

3.1.3. Factors related to semen

The OCR in cows inseminated with semen of known good quality was significantly higher (50.7%, p <0.05) than that in cows inseminated with semen of unknown (and therefore uncertain) quality. Thawing of semen in warm water or in air yielded significantly (p <0.05) higher CR than thawing in cold water.

3.1.4. Factors related to AI technique and the technician

The OCR was significantly higher (40.2%) and the number of services per conception was lower (2.5) in cows with easy passage of the AI gun through the cervix than in those with difficult passage of the gun (17.4% and 5.8, respectively).

The FSCR and OCR were significantly higher (p <0.05) in cows inseminated by holders of professional degrees (35.8% and 48.7%, respectively) compared with those in cows inseminated by holders of diplomas or high school certificates.

3.2. Monitoring the success of AI through progesterone analysis

The concentration of progesterone was determined in 149 milk samples collected on day 0 (day of AI), 140 collected on days 10–12 and 103 collected on days 21–23. The cows were categorized as having either low (<1 nmol/L), intermediate (1–3 nmol/L) or high (>3 nmol/L) milk progesterone concentration on a particular day.

On day 0, 76 cows (50%) had low milk progesterone concentration, indicating absence of luteal activity, 46 cows (30%) had high concentration, and 27 (18%) had intermediate concentration. The cows with either high or intermediate progesterone were clearly not in proper oestrus at the time of AI, being either in the luteal phase or too early or too late in the follicular phase for conception.

Based on progesterone concentrations in two milk samples taken from 140 cows on day 0 and days 10–12, 70 (50%) had low progesterone concentration in the first sample and high concentration in the second sample, indicating that they had an ovulatory oestrus. Seventeen cows (12 %) had high progesterone in both the samples suggesting that they were either in the luteal phase, were pregnant or had a luteal cyst.

Results from progesterone analysis in three milk samples (days 0, 10–12 and 20–23), followed by rectal palpation for pregnancy diagnosis 60–70 days later, were available for 103 cows (Table II). Forty-nine of these cows had a sequence of progesterone levels that were low-high-high followed by a positive pregnancy diagnosis, indicating a CR of 47.6%. Eight cows (7.8%) had a sequence of low-high-low progesterone levels followed by a negative pregnancy diagnosis, indicating fertilization failure or early embryonic death. Ten cows (9.7%) had a sequence of low-high-high progesterone levels followed by a negative pregnancy diagnosis, indicating late embryonic death, luteal cyst or persistent CL.

Intermediate concentrations of progesterone (1–3 nmol/L) were found in at least one of the three samples in 36 cows (34.9%) and they were all diagnosed as non-pregnant. This could be due to conception failure arising from improperly timed AI, suprabasal levels of progesterone at the time of AI or subsequent luteal deficiency.

TABLE II. MILK PROGESTERONE CONCENTRATIONS ON DAYS 0, 10–12 AND 20–23 AFTER INSEMINATION AND SUBSEQUENT PREGNANCY STATUS IN 103 COWS

Progesterone concentration			Pregnancy diagnosis	Number (%)
Day 0 (AI)	Day 10–12	Day 22–24		
*Low	**High	High	Positive	49 (47.6)
Low	High	Low	Negative	08 (7.8)
Low	High	High	Negative	10 (9.7)
High	High	High	Positive	0 (0.0)
Low	Low	Low	Negative	0 (0.0)
At least one sample had Intermediate concentration (1–3 nmol/L)			Positive	0 (0.0)
			Negative	36 (34.9)

*Low: <1 nmol/L/; **High: >3 nmol/L

3.3. Role of luteal inadequacy in repeat breeder cows

The mean circulating progesterone concentrations during different stages of the luteal phase of the oestrous cycle in normal and repeat breeder cows are shown in Table III. Of the twenty repeat breeder cows, 11 (55%) were diagnosed as having early luteal deficiency. The mean progesterone concentration in these animals was 1.71 ± 0.89 nmol/L, which was significantly lower ($P < 0.05$) than that of control cows (5.05 ± 1.30 nmol/L).

Two animals (10%) showed significantly lower ($P < 0.05$) progesterone concentration (1.43 ± 0.92 nmol/L) than control animals (9.34 ± 4.00 nmol/L) during mid-luteal phase (days 7–12 of the cycle). In one repeat breeder cow progesterone remained lower than that of normal cows during the early- and mid-luteal phases. In six repeat breeders (30%) progesterone concentration remained <4.77 nmol/L throughout the luteal phase and was significantly ($P < 0.05$) lower than that in control cows.

TABLE III. MEAN PLASMA PROGESTERONE CONCENTRATIONS DURING DIFFERENT STAGES OF THE LUTEAL PHASE IN REPEAT BREEDING AND NORMAL CATTLE

Stage of luteal phase	Mean progesterone (nmol/L)		Repeat breeders with luteal deficiency	
	Repeat breeders	Normal cows	Number	Percentage
Early (up to day 6)	1.71 ± 0.89	5.05 ± 1.30	11	55
Mid (days 7–12)	1.43 ± 0.92	9.34 ± 4.01	2	10
Early and Mid (up to day 12)	1.52 ± 0.38	7.15 ± 0.80	1	5
Throughout the luteal phase (up to day 18)	3.65 ± 3.12	6.52 ± 3.27	6	30

3.4. Relationship between repeat breeding and prolonged oestrus

In the repeat breeding cows with a history of prolonged oestrus (Group I) the mean duration of oestrus was 3.77 ± 0.87 days (range 40–120 h), which was significantly longer ($P < 0.05$) than that in the control animals of Group II (12–36 h). The BCS of animals in Group I (2.0–2.5) was lower compared with that of animals in Group II (3.0–4.0).

Although it was not possible to determine the exact time of ovulation in the present study, the approximate day of ovulation was estimated based on the onset and end of oestrus, subsequent presence of a CL and rise in plasma progesterone concentration, which typically occurs around day 6 after the end of oestrus. The estimated time of ovulation in Group I animals was 59.72 ± 2.41 h after onset of oestrus, which was later than that in Group II animals (29.0 ± 1.85 h after onset of oestrus).

Based on the plasma progesterone concentration on the day of standing oestrus or insemination, Group I animals could be separated into two subgroups. One subgroup ($n = 10$) had a mean basal progesterone concentration of 0.47 ± 0.29 nmol/L, which was not different ($P > 0.05$) to that in Group II animals (0.47 ± 0.16 nmol/L), whereas the second subgroup ($n = 12$) had a significantly higher ($P < 0.05$) mean basal progesterone concentration (2.57 ± 1.97 nmol/L), which we designated as "suprabasal levels". Three of these animals had remnants of a CL from the previous cycle, which may have been the source of progesterone resulting in suprabasal levels.

4. DISCUSSION

4.1. Identification of factors affecting the success of AI

The CR recorded in the present study was similar to that reported earlier in India [1]. However, it is much lower than the 71% CR reported in Norwegian dairy cows [5]. Even in certain developing countries in Asia, high CRs above 60% have been reported [6]. The low CR found in the present and previous studies indicate serious problems in the operation of field AI programmes in India.

4.1.1. Factors related to the cow

The CR was higher in cows that were inseminated when they were in standing oestrus, than in cows that had shown only secondary signs of oestrus. This is similar to the findings of Dzung *et al.* [6], although some studies have observed no statistical differences in CR following inseminations done during standing heat or when only secondary heat signs were seen [7]. Generally, 'standing to be mounted' has been recorded as the most reliable period for AI [8]. The poor CR observed in the present study could have been due to a high number of AIs being done at the improper time.

The finding that cows with marked vulval swelling at the time of AI had a higher CR than those with no swelling is in agreement with Shamsuddin *et al.* [9], although Than Hla *et al.* [10] observed no significant effect of vulval swelling on fertility. The vulva becomes oedematous due to increased vascularity under the influence of oestradiol [11]. Commencing during prooestrus and being most pronounced during oestrus, the vulval mucosa becomes congested and bright red in colour [12]. Thus our findings indicate that cows with marked vulval swelling were in the appropriate stage of oestrus for AI.

Our results on the effect of the type of mucus discharge on CR are in agreement with previous findings [6, 10]. Normal uterine secretions and cervico-vaginal discharges are important for viability and transportation of sperm and ova to the site of fertilization [13]. Turbidity of uterine secretions is usually due to inflammatory exudate or altered uterine secretions [14] and is indicative of uterine pathology. Clear mucus at time of AI appears to be a pre-requisite for conception.

The type of calving experienced by a cow at the previous parturition had a marked effect on CR. As reported by Bhosrekar [15] and Stefan *et al.* [16], cows having dystocia often develop persistent endometritis, which leads to repeat breeding. This could be the reason for our findings.

Cows that had marked uterine tone when inseminated had higher CR than those with slight or no uterine tone, which is in agreement with previous findings [6, 10]. Slight or no uterine tone may indicate that the cow is not at the proper stage of oestrus, or that some uterine pathology is present. AI in such cows would clearly lead to conception failure.

4.*1.2. Factors related to farm management*

The method of heat detection used by farmers (visual or teaser) did not have a significant effect on CR in the present study. The farms were mainly small to medium scale, and were therefore under better vigilance with individual attention of the farmers. King *et al.* [17] also reported that difficulties in oestrus detection occur due to human error rather than problems with the cows. In large farms, oestrus detection with a teaser becomes a necessity, as visual observation on each animal is not possible. For best results, however, teasers should be used in combination with visual observation [18].

Our findings on the effects of the time interval between the first signs of heat observed by a farmer and the insemination on CR are in accordance with previous studies, which reported the best CR following AI after 15–24 h [6]. As a general rule, the most appropriate time for AI appears to be 15–23 h after the onset of oestrus.

The type of housing appeared to have no significant effect on CR, although cows in tie stalls appeared to have slightly higher CR. They belonged to farmers having fewer numbers of cows and therefore received more individual care. Although Toleng *et al.* [19] observed significantly higher CR in cows kept in paddocks compared with other housing systems, the findings of Wattiaux [20] suggest that poor management and quality of housing, rather than the type of housing, has greater influence on fertility.

4.1.3. Factors related to semen

Our finding that semen of good quality gave higher CR than semen of unknown quality indicates that the latter was probably of poor quality. Failure to undertake routine examination of the semen stock in the field is therefore likely to result in low CR. A majority of the repeat breeding problems are possibly due to this factor, as the quality of semen influences both fertilization rate and embryonic mortality [21].

The importance of correct thawing of frozen semen in warm water at 35–37°C is well-documented [22]. Despite that, of the 214 observations on thawing procedure, 45 were done in cold water. Our results clearly show that higher CR is obtained when thawing is done in warm rather than cold water. Therefore, education of the inseminators on proper semen thawing procedures is essential.

4.1.4. Factors related to AI technique and the technician

Difficult entry of the AI gun into the cervix could indicate that the animal is not in proper oestrus or has some pathological condition, such as infantile genitalia or an abnormal cervix. It may also be a reflection on the lack of expertise of the inseminator.

The present study showed that the level of education of AI technicians had direct bearing on CR, as was reported by others [19]. This suggests that technical training in AI and knowledge of reproductive anatomy and physiology are important for the success of AI, as opined also in previous studies [23].

4.2. Monitoring the success of AI through progesterone analysis

In the present study we used the threshold progesterone values of <1.0 nmol/L and >3.0 nmol/L in skim milk to determine the absence or presence of luteal activity, respectively. These are in accordance with the recommendation in AIDA and have been validated through previous studies [6, 10, 18].

Our finding that nearly 50% of the cows had high or medium progesterone concentration on the day of insemination (day 0), indicates that these animals were inseminated at inappropriate times. The cows with high progesterone were either in the luteal phase, had partially luteinized cysts or were in gestational oestrus. Chauhan *et al.* [24] reported an occurrence of 20.3% gestational oestrus among crossbred cows in Punjab. Claus *et al.* [25] reported that the incidence of inseminations during luteal phase were 5.2% on experimental farms and 21.3% in problem herds under field conditions. The cows with intermediate progesterone were either too early or too late in relation to oestrus. Detection of oestrus is still a major problem and errors in detection are known to account for a large proportion of repeat breeder cows [26].

Results from progesterone concentration in two milk samples taken on day 0 and day 10–12 after AI also indicated that a high a high percentage of inseminations were done on cows that were either anoestrous, at an inappropriate stage of the oestrous cycle, pregnant, or had luteal cysts. Similar results have been reported from other countries in Asia [23] and Latin America [7].

Interpretation of the reproductive status of an individual cow at the time of AI and the subsequent outcome was possible by combining the results from all three milk samples (days 0, 10–12 and 20–23) and manual pregnancy diagnosis. Thus, 47.6% of the cows had a combination of low-high-high progesterone followed by positive pregnancy diagnosis, indicating successful pregnancy. A low-high-low progesterone combination with negative pregnancy diagnosis was found in 7.7% of cows, indicating conception failure or early embryonic death, while a low-high-high progesterone combination with negative pregnancy diagnosis was found in 9.7%, indicating late embryonic death, luteal cyst, persistent CL or prolonged cycle. Claus *et al.* [25] found that late embryonic mortality occurred in 10–17% of inseminated cows, while Lamming and Darwosh [27] found that prolonged luteal phases occurred in 8% of cows. In the present study intermediate progesterone values were found in at least one of the three samples from 34.9% of non-pregnant cows, indicating that conception failure could have been due to either improperly timed AI, suprabasal levels of progesterone at the time of AI or subsequent luteal deficiency.

4.3. Role of luteal inadequacy in repeat breeder cattle

Our finding that mean plasma progesterone concentrations were lower during one or more stages of the luteal phase in 45% of the repeat breeding cows, when compared with that in normal cows, indicates that this may be an important cause of infertility. The highest incidence of embryonic mortality is around 6–8 days after insemination, when the embryo enters the uterus and blastocoele formation takes place [28]. Inadequate luteal function during the early stages may be of prime importance because normal embryonic development depends upon sequential changes in uterine secretions that occur under the influence of progesterone [29]. It has been suggested that luteal inadequacy due to a diminished response to circulating LH may account for early embryonic mortality leading to the repeat breeding syndrome [30]. Larson *et al.* [31] postulated that delay in the onset of the luteal phase could be associated with decreased fertility in dairy cows.

Previous work has also indicated that insufficient release of progesterone during the early and mid luteal phases may cause repeat breeding [32]. Kasrija [33] observed that milk progesterone levels were deficient in 5% of repeat breeder cows during the mid luteal phase (days 6–12 of the oestrus cycle), in 10% during the late luteal phase (days 12–16) and in 15% throughout the luteal phase. Kimura *et al.* [28] suggested that a delay in the rise of milk progesterone during the early phase or levels below 6.36 nmol/L during the mid and late phases indicate luteal dysfunction. Our findings confirm that disturbances of luteal function, indicated by low plasma or milk progesterone concentration, are associated with decreased fertility in cattle.

4.4. Relationship between repeat breeding and prolonged oestrus in cattle

Prolonged duration of oestrus, which was observed in many repeat breeding cows during the present study, has also been reported in previous studies [34]. The tendency for this occurring more frequently in heifers indicates that nutrition may have a role, through negative energy balance [35]. The time of ovulation in the animals showing prolonged oestrus appeared to be delayed when compared with that in animals with a normal duration of oestrus. Delayed ovulation has been defined as failure to ovulate within 36 h after the onset of oestrus or 24 h after insemination [36]. Singh [37] has shown that the interval from onset of oestrus to ovulation is increased (53.3 ± 2.7 h) in animals with prolonged oestrus.

Three cows with suprabasal levels of progesterone had remnants of a CL from the previous cycle, which may have been the source of progesterone. Elucidation of the possible role of incomplete luteolysis of the CL of a previous cycle in the causation of suprabasal progesterone levels during the subsequent oestrus period needs further investigation. The nine animals with suprabasal progesterone levels that had no remnant of a CL possibly had other sources of progesterone. Bage *et al.* [38] studied the effect of ACTH on ovariectomised repeat breeding heifers to ascertain whether the adrenal glands, responding to stress during oestrus, could be a source of extra-gonadal progesterone, but the results were not conclusive. The suprabasal progesterone concentrations might also be a cause of prolonged oestrus [34]. Rising oestradiol during the follicular phase triggers the pre-ovulatory surge of LH only if the concentration of progesterone is low [39]. High progesterone concentration inhibits the LH surge [40], while exogenously maintained suprabasal progesterone during oestrus decreases the magnitude of the surge [41]. These changes prevent or delay ovulation, causing the dominant follicle to continue its growth, reaching a larger size and secreting greater amounts of oestradiol for a longer period than is normal [42]. This can lead to prolonged duration of oestrus. The present study indicates that suprabasal progesterone levels during oestrus may be an important factor in the causation of prolonged oestrus, which results in repeat breeding, but the therapeutic interventions that could overcome this problem need to be investigated further.

In conclusion, the present study has identified serious drawbacks in the AI programme currently operated at the field level in India, which resulted in low CR. The major lacuna is the lack of adequately trained personnel to perform AI on small and medium livestock farms. The AIDA software program is a tool that is effective for the management and analysis of AI data. Two important findings of the study were that luteal insufficiency at different stages of the luteal phase and suprabasal progesterone levels during oestrus are important factors contributing to the failure of AI in some cows. These need further detailed investigations to formulate remedies. A follow-up study was conducted to test several interventions aimed at improving the fertility of cows subjected to AI under field conditions and the results are reported separately in this publication [43].

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INTERVENTIONS FOR IMPROVING THE FERTILITY OF CROSSBRED COWS SUBJECTED TO ARTIFICIAL INSEMINATION UNDER FIELD CONDITIONS

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Abstract

INTERVENTIONS FOR IMPROVING THE FERTILITY OF CROSSBRED COWS SUBJECTED TO AI UNDER FIELD CONDITIONS

As a follow-up to a study that assessed the various factors affecting conception rate in cows bred by artificial insemination (AI), several interventions were tested for their effectiveness in overcoming the major problems that were identified. Treatment of repeat-breeder cows that were diagnosed as having sub-clinical endometritis with 100 µg *E.coli* lipopolysachharide (LPS) or LPS + autologous serum resulted in reduced incidence of abnormalities in the appearance and consistency of cervico-vaginal mucus (CVM) and a significant decrease in pH of CVM from around 7.7 to 7.2. Treatment of repeat-breeder cows that were suspected of having luteal deficiency with two injections of 500 mg progesterone on days 5 and 11 after AI resulted in 30% of the animals conceiving compared with 20% conception in controls. Filtration of frozen and thawed bull semen through Sephadex columns significantly decreased the concentration of dead and abnormal sperms. The CR in cows inseminated with filtered semen was 57%, compared with 22% in cows inseminated with unfiltered semen. A number of refresher training courses were held for reproduction scientists, field veterinarians and farmers on the causes of infertility and to introduce the latest reproductive technologies for improving fertility in dairy animals.

1. INTRODUCTION

Normal fertilization, early embryonic development and maintenance of pregnancy are influenced by the environment inside the uterus [1], quality of semen [2] and increasing progesterone concentration that occurs between 4–10 days after ovulation [3]. The uterine environment is adversely affected by bacterial endometritis, which alters the physical properties of the cervico-vaginal mucus (CVM) [4]. The quality of frozen thawed semen used in artificial insemination (AI), and consequently its fertilizing capacity, is reduced by the presence of high numbers of dead and abnormal sperms [2]. A hostile uterine environment and poor semen quality result in fertilization failure and deficiency of progesterone during the early stages of embryo development causes death of the embryo. All three factors lead to decreased fertility.

The use of intrauterine antibiotics and antiseptics for treatment of endometritis has produced variable results [5]. The disadvantages of such treatment are the cost, need to withhold milk from sale or consumption, development of microbial resistance and inhibition of the phagocytic activity of polymorphonuclear cells [6]. Therefore, the first intervention tested in this study was the use of lipopolysachharide (LPS) from *Escherichia coli* for treating sub-clinical endometritis.

Various clinical trials in cattle have been conducted to study the effect of progesterone supplementation after AI on pregnancy rates, but the results have been conflicting [7]. The second intervention tested in the present study was the supplementation of repeat-breeding cattle with progesterone after AI. The third intervention tested the effects of improving the quality of frozen thawed semen by Sephadex filtration on fertility of inseminated cows.

The overall objectives of these studies were: (a) to develop appropriate interventions to improve fertility; and (b) extend their use through refresher training of scientists, veterinary officers and dairy farmers.

2. MATERIALS AND METHODS

2.1. Effect of *E. Coli* LPS in cows with sub-clinical endometritis

Thirty-six crossbred repeat-breeder cows were included in the study. Sub-clinical endometritis was confirmed on the basis of bacterial culture and isolation from uterine swabs. The animals were divided randomly into three groups of 12 each. They were given the following intrauterine infusions 8–10 h after insemination: Group 1 (control), 20 mL phosphate buffered saline (PBS); Group 2, 100 µg of *E. coli* LPS (serotype 026:B6 containing 10 000 endotoxin units/mg of LPS, Sigma, USA); and Group 3, 100 µg of LPS + 5 mL autologous serum.

The CVM was collected using a glass pipette attached to a syringe before treatment and 12 h later. The colour of CVM was assessed by naked eye and the consistency was graded as thin (++) or thick (+++). The pH was determined using a pH paper strip.

2.2. Effect of progesterone supplementation in repeat-breeder cows

Thirty repeat-breeder cows weighing between 300–550 kg were used in the study. All animals had apparently normal genital organs, oestrous cycle, oestrus period and CVM, but had failed to conceive after 4–8 consecutive inseminations with good quality semen. They were divided into two groups: Group 1 (n=20) were not treated (controls); and Group 2 (n=10) were treated with 500 mg of 17-α-hydroxy-progesterone caproate (Duraprogen, Unichem Agvet, Mumbai, India), injected intramuscularly on the fifth and eleventh days after insemination.

The cows were examined for oestrus twice daily starting from two days before the expected date of onset of oestrus by parading vasectomized teaser bulls. The CVM was examined microscopically for fern pattern. All animals were inseminated at standing oestrus (designated as day 0) with good quality frozen thawed semen. Pregnancy diagnosis was done by per-rectal examination 60 days after AI.

Milk samples were collected from each animal on days 0, 4, 6, 8, 12, 16 and 20 or 22 after AI into tubes containing sodium azide as a preservative, the fat was removed and the skim milk was stored at -20°C. Progesterone was measured by radioimmunoassay (RIA) using kits (Diagnostic Products Corporation, USA) containing progesterone antibody pre-coated tubes and pre-diluted ^{125}I progesterone tracer, according to the protocol provided by the IAEA.

2.3. Effect of Sephadex filtration on quality and fertility of semen

Bull semen that had been frozen in French straws (0.5 mL) was used for the study. Semen samples from each bull were thawed and used for AI either without Sephadex filtration (Group 1, control) or after filtration (Group 2, treatment). The detailed procedure was as follows.

2.3.1. Preparation of Sephadex columns

Columns of Sephadex G-15 (particle size 40–120 microns, Pharmacia Fine Chemicals, Uppsala, Sweden) were prepared according to the method described by Graham *et al.* [8]. A slurry of Sephadex (20% W/V) was prepared in 3% sodium citrate solution (pH 6.8) and allowed to swell in the refrigerator for at least three hours. Sterilized glass wool (100 mg) was placed at the bottom of a 5mL sterilized glass syringe and the Sephadex slurry was added to prepare a column of 0.6 mL. The syringes containing the columns were stored at 5°C until used.

2.3.2. Filtration of semen

Semen was thawed and filtered just before use in AI. The syringes containing Sephadex columns were placed in an incubator at 37°C for 30 min and the columns were wetted by adding 3–4 drops of 3% sodium citrate solution. For each insemination, two straws of frozen semen from the same bull were thawed at 37°C for 30 sec and the semen was gently placed over the Sephadex column. Filtered semen was collected in a 2 mL Eppendorf tube. The filtration process was done at 37°C with protection from bright light and drafts, and was completed within 2–3 min.

2.3.3. Examination of semen

Thawed semen samples, both unfiltered and filtered, were subjected to the following tests: (a) total sperm concentration was measured using the Neubaur chamber method; (b) individual sperm motility was estimated microscopically; (c) live sperm count was done by the differential staining technique using Eosin-Nigrosin stain [9]; and (d) abnormal sperm count was done using Rose Bengal stain [10]. The concentration of motile, live and abnormal sperms was calculated for each sample.

2.3.4. Fertility trials

The fertility of unfiltered and filtered semen samples was tested on 62 healthy, normally cycling crossbred (Holstein-Friesian x Sahiwal) cows. Oestrus detection was performed by using vasectomized bulls and then confirmed by per-rectal palpation of genitalia. Cows were divided randomly into two groups and cows in Group 1 (control, n=32) were inseminated with unfiltered semen, while cows in Group 2 (treatment, n=30) were inseminated with Sephadex filtered semen.

For cows in Group 1, two straws of thawed semen were pooled in a 2 mL Eppendorf tube, 0.5 mL was aspirated into an empty sterilized straw using an adapter attached to a syringe and AI was done within 5 min of pooling. For cows in Group 2, 0.5 mL of filtered semen was aspirated in to an empty straw and used for AI. Each cow was inseminated twice, with an interval of 12 h, during standing oestrus.

The cows were kept under observation for return to heat and those not returning were checked for pregnancy by per-rectal palpation two months after AI.

2.4. Statistical analysis

The results were statistically analyzed where required using Student's t test.

2.5. Training and extension

During the course of the project several training and extension activities were held for scientists from other State Agricultural Universities, field veterinary officers, AI technicians and dairy farmers. The topics included various aspects of dairy farming, including management, economics, reproduction, AI, infertility and the application of the interventions that were tested during this study.

3. RESULTS

3.1. Effect of *E. Coli* LPS in cows with sub-clinical endometritis

The CVM was turbid before treatment in 66%, 75% and 80% of animals in Groups 1, 2 and 3, respectively. After treatment it remained turbid in the same proportion of animals in Group1 (control, PBS), but was significantly reduced ($P < 0.05$) to 25% and 16% in Groups 2 (LPS) and 3 (LPS + plus autologous serum), respectively.

The consistency of CVM before treatment was thick (+++) in 96% of animals in each group. After treatment it remained thick in animals of Group 1, but changed to thin (++) in 90% of animals in Groups 2 and 3 ($P < 0.05$). The pH of CVM before treatment was around 7.7 in all groups. After treatment it remained unchanged in Group 1, but decreased to 7.2 in Groups 2 and 3.

Bacteria were isolated from the uterine swabs of all the cows. The highest numbers of isolates were *Escherichia coli, Staphylococcus aureus* and *Proteus* species. Other relatively less common isolates were species of *Streptococcus*, *Klebsiella, Pseudomonas, Corynebacterium* and *Bacillus*.

3.2. Effect of progesterone supplementation in repeat-breeder cows

In Group 1 (untreated) 4 out of 20 cows (20%) became pregnant. The other 16 cows returned to oestrus 20–22 days after AI. In Group 2 (treated with progesterone) 3 out of 10 cows (30%) became pregnant, but the difference was not significant. Progesterone concentration after AI in cows of Groups 1 and 2 are presented in Table I. Untreated cows had lower progesterone concentration than treated cows on several days of sampling, but the differences were significant only on three occasions.

3.3. Effect of Sephadex filtration on quality and fertility of semen

The characteristics of frozen and thawed bull semen with and without Sephadex filtration are given in Table II. Filtration significantly ($p < 0.05$) decreased the average total sperm concentration and the number of sperms with abnormal head, mid piece and tail. The procedure significantly ($p < 0.05$) increased the average concentrations of motile and live sperms. The average CR in cows inseminated with unfiltered semen was 21.9%, while that in cows inseminated with filtered semen was 56.7% ($p < 0.05$).

3.4. Training and extension

A summary of the training and extension activities conducted for improving the knowledge and skills of scientists, veterinarians and farmers on various aspects of dairy farming is given in Table III.

TABLE I. MEAN (± S.E.) PROGESTERONE CONCENTRATIONS IN SKIM MILK OF REPEAT-BREEDER COWS WITH OR WITHOUT PROGESTERONE TREATMENT

Group	Result	Progesterone concentration (nmol/L) on days after insemination						
		0	4	6	8	12	16	20–22
Untreated	Pregnant (n=4)	0.16 ± 0.03	2.73 ± 0.03	4.04 ± 0.29	5.21 ± 0.25	7.06 ± 0.57	7.89 ± 0.32*	8.97 ± 0.41
	Non-pregnant (n=16)	1.18 ± 0.29	4.49 ± 0.25	2.67 ± 0.25	3.34 ± 0.29*	4.45 ± 0.47*	5.79 ± 0.73	0.73 ± 0.19
Treated	Pregnant (n=3)	0.22 ± 0.06	1.84 ± 0.13	4.77 ± 0.29	6.42 ± 0.44	8.59 ± 0.35	9.64 ± 0.32	11.77 ± 0.38
	Non-pregnant (n=7)	0.89 ± 0.73	2.35 ± 0.22	3.78 ± 0.51	4.77 ± 0.31	6.39± 0.32	3.78 ± 0.45	2.23 ± 0.67

*Significantly lower than the value in corresponding treated groups ($P < 0.05$).

TABLE II. CHARACTERISTICS OF FROZEN THAWED BULL SEMEN WITH AND WITHOUT FILTRATION THROUGH SEPHADEX COLUMNS (N=34)

Characteristic	With filtration (millions/mL)	Without filtration (millions/mL)
Total sperms	53.4 ± 1.70*	80.4 ± 0.96
Motile sperms	38.8 ± 2.03*	29.2 ± 1.11
Live sperms	38.0 ± 2.20*	32.0 ± 0.96
Head defects	1.2 ± 0.82*	2.4 ± 0.23
Mid-piece defects	1.3 ± 0.88	2.3 ± 0.23
Tail defects	6.4 ± 1.18*	15.7 ± 0.54

*Values differ significantly ($p < 0.05$) from those without filtration

4. DISCUSSION

4.1. Effect of *E. Coli* LPS in cows with sub-clinical endometritis

The CVM discharged in healthy animals during oestrus is usually clear and colourless [11]. Discharge of cloudy, milky or turbid CVM is indicative of uterine or cervical infections [12]. The consistency of CVM is usually thin or watery in normal bovines. In earlier studies, the incidence of thin CVM in repeat-breeder cows was either similar to [14, 15] or lower [12] than in normal animals. Changes in CVM occur due to microbial infection and variations in appearance or consistency may be due to differences in the cause and type of inflammation [16]. The consistency of CVM depends primarily on the degree of cross-linkage in the epithelial glycoprotein macromolecules of oestrual cervical mucus, which in turn influences the penetrability of the cervical canal to spermatozoa [17]. The pH of normal CVM is around 7.0–7.2 and that of oestrual CVM in repeat-breeder cows with sub-clinical endometritis ranged from 7.4 to 8.0 [12].

In the present study, treatment with LPS or LPS + serum resulted in reduced incidence of abnormalities in the appearance and consistency of CVM, and a significant decrease in its pH to a mean of 7.2. It can therefore be concluded that these two treatments were effective in restoring the normal characteristics of CVM in a high proportion of animals.

Bacteria were isolated from the uterine swabs of all the cows and the type of bacteria isolated were similar to those previously reported in bovines with endometritis [13]. However, studies on repeat-breeding bovines [12] have found that other types of bacteria, such as *Actinomyces pyogenes*, *Streptococcus* spp. and *Bacillus* spp., are also frequently observed. A major cause of sub-clinical endometritis may be the unhygienic conditions under which some technicians perform AI, leading to the entry of these organisms into the uterus.

TABLE III. TRAINING AND EXTENSION ACTIVITIES CONDUCTED DURING THE PROJECT

Name of activity (Number of each activity)	Type of trainees	Number of trainees	Duration of training
Diagnostic techniques for reproductive problems in bovines (1)	Scientists from universities	12	21 days
AI under field conditions (1)	Veterinarians	6	One day
Infertility in dairy animals (1)	Dairy farmers	32	10 days
Ideal management of dairy animals (1)	Dairy farmers	35	10 days
Improving fertility in dairy animals (4)	Dairy farmers	30–35 each	One day each
Bottlenecks in field AI programmes (4)	Veterinarians	7–10 each	One day each
Hints on commercial dairy farming (2)	Dairy farmers	50 each	One day each
Improvement of fertility through integrated management practices (1)	Scientists from universities	10	21 days
Educational talks on animal health (2)	Dairy farmers	10 each	One day each
Economic benefits of commercial dairy farming (2)	Dairy farmers	60 each	One day each
Talks on reproductive management practices in villages (6)	Dairy farmers	30–50 each	One day each
Refresher training courses on improving the success of field AI (8)	Veterinarians	6–12 each	One day each
TV talk on factors affecting the success of AI (1)	Viewers in North India	-	30 minutes
Management of infectious infertility in domestic animals (1)	Scientists from universities	11	21 days

4.2. Effect of progesterone supplementation in repeat-breeder cows

The improvement in CR after progesterone therapy in our study was similar to that in a previous report [18]. However, other workers have reported either no significant increase [19] or a decrease [20] in CR after such treatment.

The slightly higher progesterone concentrations found in the milk of treated cows could be due to a cumulative effect of the exogenously administered progesterone supplementation. Previous studies have reported no significant effect of progesterone administration at rates of 100 mg daily between days 10 and 20 after AI [21], or 200 mg on days 5, 7, 9 and 11 after AI [22] on plasma progesterone levels.

4.3. Effect of Sephadex filtration on quality and fertility of semen

Sephadex filtration of frozen thawed semen significantly reduced the total sperm concentration, increased the proportions of motile and live sperms and decreased the proportion of abnormal sperms. Similar observations have been reported in previous studies [8, 23–25]. The increase in the proportion of motile and live sperm is due to retention of dead, immotile and abnormal sperms in the Sephadex column [8].

The overall average CR with filtered semen was significantly ($p < 0.05$) higher (56.7%) than that with unfiltered semen (21.9%). The process of freezing and thawing semen causes damage to a proportion of the sperms [26, 27] and reduces CR when compared with that achieved using fresh semen [28]. Damage to the plasma membrane of sperm stimulates the production of reactive oxygen species [29], which affects its permeability and DNA strand configuration [30]. Moreover, toxic effects are enhanced in the presence of egg yolk, which is an ingredient used in the diluent for freezing sperm [31]. Sephadex filtration may have an additional advantageous effect by reducing the concentration of egg yolk and yielded sperms with higher acrosin activity [32]. It can therefore be concluded that Sephadex filtered semen has a higher proportion of viable and competent spermatozoa to facilitate fertilization of the ovum and can increase the CR in cattle.

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IMPROVEMENT OF THE EFFICIENCY OF ARTIFICIAL INSEMINATION SERVICES THROUGH THE USE OF RADIOIMMUNOASSAY AND A COMPUTER DATABASE APPLICATION

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Abstract

IMPROVEMENT OF THE EFFICIENCY OF ARTIFICIAL INSEMINATION SERVICES THROUGH THE USE OF RADIOIMMUNOASSAY AND A COMPUTER DATABASE APPLICATION

A study was conducted at several locations in four provinces of Indonesia to evaluate and increase the efficiency of artificial insemination (AI) services provided to cattle farmers and to improve the feeding and reproductive management practices. Radioimmunoassay (RIA) for progesterone measurement was used together with the computer program Artificial Insemination Database Application (AIDA) to monitor the success of AI and for the early diagnosis of non-pregnancy and reproductive disorders in dairy and beef cattle. Baseline surveys showed that the average calving to first service interval (CFSI) ranged from 121.3 ± 78.2 days in West Java to 203.5 ± 118.3 in West Sumatra, and the conception rate (CR) to first AI ranged from 27% in South Sulawesi to 44% in West Java. Supplementary feeding with urea-molasses multi-nutrient blocks (UMMB) combined with training of farmers on improved husbandry practices reduced the CFSI from 150.6 ± 66.3 days to 102.3 ± 36.5 days and increased the CR from 27% to 49% in South Sulawesi. Similar interventions in West Java reduced the CFSI from 121.3 ± 78.2 days to 112.1 ± 80.9 days and increased the CR from 34% to 37%. Results from measurement of progesterone in milk or blood samples collected on days 0, 10–12 and 22–24 after AI showed that 25% of the animals were non-

cyclic or anovulatory, while 8.7% were pregnant at the time of AI. Investigation of cows with breeding problems using measurement of progesterone in combination with clinical examination revealed a range of problems, including true anoestrus, sub-oestrus or missed oestrus, persistent CL and luteal cysts. The ability to make an accurate diagnosis enabled the provision of appropriate advice or treatment for overcoming the problems. Anti-progesterone serum and ^{125}I-Progesterone tracer for use in RIA were produced locally and were found to have acceptable characteristics. The tracer had good specific activity and stability for up to 12 weeks. The production of standards in skim milk needs improvement and the RIA requires further work for optimization and validation.

1. INTRODUCTION

Within the last decade, agriculture has become the backbone for economic recovery of Indonesia after the monetary crisis that occurred in 1997. In this context, livestock production forms an integral component of agriculture, with dairy and beef cattle being of high importance. In cattle farming, national development efforts are aimed at improving nutrition, reproductive management and health care.

Consumption of beef in 1997 was 1.9 kg/capita/year and is expected to rise to 3.5 kg/capita/year within ten years. Currently, the national demand for beef is estimated at 498 000 tonnes, of which 74% is supplied from domestically slaughtered cattle. The shortfall is expected to be met through three major efforts: intensification of the fattening of local male cattle resulting from artificial insemination (AI), fattening of imported cattle and importation of high quality beef for the exclusive market. The demand is expected to increase annually by 7.8%, due to increases in population, national economic growth and per capita income. However, livestock production is constrained by poor nutrition, both in quantity and quality, and poor reproductive performance. In practical terms, integration of all aspects of the production system in large ruminants is necessary to achieve improvements.

The main dairy cattle farming system in Indonesia is small scale, with each farmer keeping only 3–4 animals. There are many factors to be considered when attempting to increase the productivity of such farms. One important constraint to higher productivity is low reproductive rate. It has been recognized that establishing a system of data recording and its use for management decisions is an important prerequisite to improve the reproductive rate. This requires collaboration between the farmers and their service providers, i.e. AI technicians and veterinary assistants. Therefore, in parallel with training given to the farmers on reproductive management of dairy cattle, a comprehensive but simple recording system that can be used by field technicians is necessary. Such a system should provide information that assists in the early detection of reproductive disorders in dairy cattle, so that the local veterinarian can be informed. The latter, in turn, can use the information together with a clinical examination to arrive at a diagnosis, and then provide the necessary treatment to the cow or advice to the farmer.

Progesterone is a reproductive hormone produced by the corpus luteum (CL) in the ovary and the placenta of female cattle. The concentration of this hormone in the blood and milk can be measured using radioimmunoassay (RIA), and the information can be used to predict the reproductive status of a cow and to diagnose certain conditions or disorders that result in sub-fertility [1]. This information is particularly useful when combined with other physiological data relating to calving, oestrus and inseminations. A study was therefore undertaken to institute a field data recording system, using a computer program entitled Artificial Insemination Database Application (AIDA) that has been designed for this purpose by the Joint FAO/IAEA Division of Nuclear Techniques, Vienna [2]. This was used in combination with measurement of progesterone in samples of milk or blood of cattle, to monitor the effectiveness of the AI programme and for early diagnosis of non-pregnancy and sub-fertility. The study was carried out in different locations in Indonesia, using pilot farms to initially validate the procedure and to subsequently serve as demonstration sites.

The objectives of the project reported in this paper, which are complementry to the national priorities for research and development, were to: (a) perform surveys on the AI services offered to small holder farms in selected provinces, in order to identify the factors that limit the success rate of AI; (b) develop and implement appropriate remedial measures to enhance the efficiency of the AI services; and (c) develop and establish the production of RIA progesterone kits in Indonesia through the National Nuclear Energy Agency (NNEA).

2. MATERIAL AND METHODS

2.1. Survey on reproduction of cattle bred by AI

Before commencing the baseline survey on the reproductive pattern of native and crossbred cattle bred by AI, the recording system using AIDA was introduced and implemented in several locations of West and Central Java, South Sulawesi, and West Sumatra. The tables for recording field data were translated in to Bahasa Indonesia. Training was conducted for the field veterinarians, extension workers and technical staff of the district Livestock Services, staff of collaborating universities, members of dairy cooperatives and individual farmers. The training was in the form of workshops designed to introduce the application of AIDA and RIA progesterone techniques in order to improve on going AI programmes through monitoring and identification of constraints. Further training was conducted for staff of the main collaborating institutes on data entry using the AIDA software and on routine operation of the application, and for field veterinarians and technical staff on identification of sub-fertility and infertility and the introduction of interventions to overcome them.

2.1.1. Locations and methodology

The field survey was conducted in four provinces of Indonesia, in collaboration with the provincial universities and the district Livestock Services. Table I shows the provinces, main collaborative institutes, types of cattle production systems and the target numbers of cows included in the survey.

The methodology was according to the guidelines provided in the AIDA protocol [2]. Cows presented to AI were recorded and followed until they became pregnant. On the day of the first AI service (day 0), information relating to the farm, cow, AI technicians and semen batch were recorded and a sample of blood (beef cattle) or milk (dairy cattle) was collected for progesterone RIA. The farms were revisited on day 10–12 after AI to collect a second sample, and on day 20–22 to collect a third sample. Further data was recorded from cows that returned to repeat AI services and pregnancy diagnosis was performed by rectal examination 50–90 days after the last service. Any modifications to this schedule or other specific studies including interventions done in the different locations are described below.

Plasma was separated from the blood samples and skim milk was separated from the milk samples; they were stored at –20°C until assayed. All samples were assayed for progesterone by the 'Self-coating RIA' method using the reagents and protocol provided by the Joint FAO/IAEA Division of Nuclear Techniques, Vienna. The method is a direct solid-phase RIA, employing a monoclonal antibody, ^{125}I-progesterone as tracer and standards ranging from 0 to 40 nmol/L prepared in skim milk. Data were recorded and plotted using the Grafit software programme. The intra-assay and inter-assay coefficients of variation were 5.6% and 8.9%, respectively.

Data interpretation was based on the concentration of progesterone in one or a series of samples, as outlined in the AIDA protocol [2] and the observations reported by Zdunczyk *et al.* [3]. Progesterone concentrations in plasma or skim milk below 1 nmol/L were considered as indicating absence of an active CL in the ovary, while concentrations above 3 nmol/L were considered as demonstrating presence of luteal activity. Progesterone concentrations in the range 1–3 nmol/L were classified as 'doubtful', meaning that a diagnosis was not possible and required either re-assay of the sample or examination of the cow by a veterinarian. In each case the interpretation of progesterone values was cross-checked with the result from rectal palpation.

Data from field AI recording and laboratory progesterone analyses were entered in the AIDA program and summary tabulations were generated. The fertility indices were examined in relation to the variables and factors recorded and those that appeared to influence reproductive performance were selected for further analysis. The relevant data was exported to MS-Excel and analysed using the Systat v.7 statistical package.

TABLE I. LOCATIONS AND RELATED INFORMATION ON THE SURVEY CONDUCTED ON CATTLE BRED BY AI

Province	Main counterpart institute	Type of cattle	Number of cows recorded
West Sumatra	University of Andalas, Padang	Native crossbred beef cattle	100
South Sulawesi	University of Hasanuddin, Makassar	Bali breed beef cattle	180
West Nusa Tenggara	University of Mataram, Lombok	Brahman breed beef cattle	75
West and Central Java	National Nuclear Energy Agency, Jakarta	Dairy cattle	260

2.1.1.1. West Sumatra

The main smallholder farming system of the province is beef cattle and AI was introduced in 1993 in order to improve productivity. However, poor recording of AI and lack of extension workers resulted in low efficiency of the AI service, requiring 3–4 services per conception. Therefore, the aim of the present study was to apply RIA and AIDA to monitor the reproductive performance of native crossbred cattle and to improve the efficiency of AI.

The translated AIDA tables were used for field recording of data, blood samples were collected on days 0, 11 and 22 after AI for progesterone measurement, and rectal examination was conducted at 60–90 days after AI to confirm pregnancy. After the baseline survey, feeding of a multi-nutrient lick block made of *saka* (residue from the sugar-cane industry) was done as an intervention.

2.1.1.2. South Sulawesi

A baseline survey on reproductive patterns of cows bred by AI had already been done in Sulawesi [4]. Therefore, during the present project a study was conducted on the effects of improved feeding on the reproductive performance of beef cattle of the Bali breed.

Cows kept by smallholder farmers (1–3 head per farm) in 2 different locations were used for the study. The cows were grazed during the day and housed in tie-stalls at night. Oestrus detection was done by observation once daily. Eighty cows from location 1 and 100 cows from location 2 were selected during their post-partum period. Initially, each animal was vaccinated against Septicaemia Epizootica (SE) and Anthrax, and was given 1 kg of a medicated Urea Molasses Multi-nutrient Block (UMMB) as a feed supplement and anthelmintic. This block was consumed over 2–3 days. Thereafter, cows in location 1 received no further feed supplement, while cows in location 2 received an un-medicated block, which was placed in the night tie-stall with access *ad libitum*.

The translated AIDA tables were used for recording data. Blood samples were collected for progesterone measurement on days 0 and 21–23 after AI and at the time when rectal examination was conducted at 60–90 days after AI.

2.1.1.3. Lombok Island, West Nusa Tenggara

The reproductive performance was monitored in 68 Brahman cattle at two locations in Lombok Island. Blood samples were collected on days 0, 10–11 and 20–21 after AI to monitor the success of AI. Pregnancy diagnosis was done by rectal palpation 60–90 days after AI. Sample processing, progesterone measurement and data interpretation were done as described above (2.1.1.).

2.1.1.4. West and Central Java

The baseline survey involved 181 farmers in 2 districts, Garut in West Java province and Yogyakarta in Central Java province, with 260 Holstein-Friesian dairy cattle. Feeding was based on cut and carried grass from communal lands and roadsides, mixed with agricultural by-products (e.g. banana stems, vegetable crop waste and corn leaves), with each cow receiving around 35–40 kg of fresh weight daily. All animals also received a concentrate mixture, given in the ratio of 1 kg concentrate per 2 kg of milk produced. After the baseline survey, each cow was fed 500 g/day of UMMB as a supplement.

The translated AIDA tables were used for field recording of data, milk samples were collected for progesterone measurement on days 0, 11 and 22 after AI and at the time when rectal examination was conducted at 50–60 days after AI.

2.1.2. Design and testing of interventions to improve the efficiency of AI

Based on the results of the baseline survey, training and educational activities were conducted to: (a) increase the competence and managerial capability of the livestock farmers; (b) improve the feeding system; (c) improve the physiological and health status of the animals; (d) increase the efficiency of the AI technicians; and (e) improve the semen quality and its handling in the field. Improvement of the feeding system included provision of UMMB as a supplement as described above for each location. The response to these interventions was monitored and recorded during the subsequent year.

2.2. Application of progesterone RIA for early diagnosis of reproductive disorders

2.2.1. Animals, locations and feeding

This study was conducted as a follow-up to the baseline survey, and included the provinces of West and Central Java and West Nusa Tenggara. Cows identified as having reproductive problems were subjected to detailed study using measurement of progesterone in serial samples of milk or blood. The animals were kept under a tie-stall system and fed about 10% of their body weight. The basal diet comprised cut and carry grass, other green forage, banana stems, sorghum and rice straw (85–90%) and concentrate mixture (10–15%), with water available *ad libitum*. The composition of the feed materials is shown in Table II. During the period of study each animal received 300 g/day of UMMB as a feed supplement.

TABLE II. PARTIAL PROXIMATE ANALYSIS OF MATERIALS USED FOR FEEDING THE COWS DURING THE STUDY

Feed material	D.M.[a] (%)	O.M.[b] (% D.M.)	C.P.[c] (% D.M.)	C.F.[d] (%)	G.E.[e] (kJ/g D.M.)
P. purpurhoides (King grass)	25.3	89.1	12.0	27.0	17.2
Leucaena leucocephala (Ipil Ipil)	28.9	91.1	26.3	20.4	18.8
Rice straw	33.1	83.6	5.5	29.6	13.4
Corn leaves	17.7	83.4	15.3	25.6	14.5
Tapioca chopped	34.3	95.5	2.9	4.0	-
Banana stems	10.0	88.2	4.2	24.6	13.4
Mixed road-side grass	17.1	85.5	14.5	30.4	13.7
Concentrate mixture ("Puri")	90.5	88.4	14.4	14.0	14.5
UMMB	81.1	82.7	27.0	7.3	18.4

[a]Dry Matter; [b]Organic Matter; [c]Crude Protein; [d]Crude Fibre; [e]Gross Energy

2.2.2. Records and measurements

The body condition score (BCS) was recorded on a scale of 1–4. Information on the farms, animals, AI technicians, semen quality and any reproductive disorders was recorded by field technicians using the translated AIDA forms. Milk or blood samples were collected once per week for two months at Garut to monitor the level of progesterone. Blood samples were collected on days 0, 10–11 and 20–21 after AI at Yogyakarta and Lombok to monitor the success of AI. Sample processing, progesterone measurement and data interpretation were done as described above (2.1.1.).

2.2.3. Economic evaluation

An economic evaluation was done on the application of milk or serum/plasma progesterone RIA for diagnosing reproductive failure in the field. Parameters recorded were: (a) cost of assaying a sample for progesterone using RIA kits produced by the NNEA; (b) total cost for assaying three samples in cows with reproductive failure; (c) costs of treatments given on the basis of a diagnosis made using the results; (d) benefit to the farmer when a reproductive problem is effectively treated, compared with no milk samples being taken and no treatments being given.

2.3. Development and production of progesterone RIA kits in Indonesia

This study involved the development of anti-progesterone antibody, preparation of progesterone tracer and standards, optimization of the reagent dilutions for use in the assay and field-testing of the NNEA progesterone RIA kit.

2.3.1. Development of anti-progesterone antibody

The procedure was based on the method of Thorell and Larsen [5]. Three rabbits were immunized by giving sub-cutaneous injections, initially with progesterone-11α-hemisuccinate and Freund's complete adjuvant (Sigma), followed by six 'booster' injections of progesterone-11α-hemisuccinate and Freund's incomplete adjuvant (Sigma) at monthly intervals. Serum was collected before each booster to test the titer of the antibody and the maximum binding of ^{125}I-progesterone tracer in the RIA.

2.3.2. Preparation and characterization of ^{125}I-progesterone tracer

Iodination of progesterone-11α-hemisuccinate-TME was done using a procedure adapted from that used by the Bombay Atomic Research Centre (BARC), India, using Chloramin-T as oxidizing agent and $Na_2S_2O_5$ as reducing agent. Purification of the labeled compound (^{125}I- progesterone-11α-hemisuccinate-TME conjugate) was carried out using Sephadex G-25 columns (PD-10, Pharmacia) saturated with 5% bovine serum albumin (BSA) solution and eluting with 0.05M phosphate buffer (pH 7.4) in a high performance liquid chromatograph (HPLC).

Each fraction of 0.5 mL was collected into separate tubes. The efficiency of labeling was calculated and the radiochemical purity was tested by paper electrophoresis on Whatman No. 1 paper in 0.05M carbonate buffer (pH 8.5) at 240 Volts for one hour. The specific activity (μCi/μg) of tracer was calculated.

Immunoreactivity (percent binding relative to total counts) and non specific binding (NSB) of the tracer were tested by RIA using 50 μL of zero standard and 50 μL of the tracer against the anti-progesterone antibody coated on Nunc-star assay tubes. The stability of the tracer was monitored from the values for radiochemical purity, immunoreactivity and NSB on six consecutive occasions over a period of 13 weeks.

2.3.3. Preparation of progesterone standards

A series of progesterone standards in skim milk was prepared using the protocol provided by the IAEA [6]. Commercially available powdered skim milk was dissolved in nanopure water. To remove any traces of milk fat that may have been present, 5 g of activated charcoal was added to 2 L of the dissolved milk powder, stirred for one hour at room temperature and incubated overnight at 4°C. The solution was centrifuged for 15 minutes at 3000 rpm at 4°C and then filtered through tissue paper to remove the charcoal. Sodium azide (0.1 g/100 mL) was added as a preservative.

An accurately weighed portion of 0.3145 g progesterone (4-Pregnene-3,20-dione, Sigma) was dissolved in ethanol to make a 10 μM progesterone stock solution. A series of standard solutions containing 1.25, 2.5, 5, 10, 20 and 40 nmol/L of progesterone was made by transferring the required volume of stock solution to a 200 mL volumetric flask, evaporating the ethanol using a flow of nitrogen gas or air, and adding the fat free milk solution up to the 200 mL mark.

2.3.4. RIA procedure and field-testing of the kit

The procedure used for the RIA was adapted from the 'Self-coating' method developed by the IAEA and described above (2.1.1.), with slight modifications. A batch of 300 polypropylene Nunc-star assay tubes was set up for one RIA kit and an aliquot of 300 µL 1:1000 anti-progesterone antiserum in carbonate-bicarbonate buffer (0.05M, pH 9.6) was pipetted into each tube. The tubes were incubated at 4^{o}C overnight, decanted and washed twice with 500 µL of 0.1% Tween-20. The subsequent procedure was similar to that described for the IAEA 'Self-coating' kit.

Samples of milk from cattle in South Kalimantan were assayed using both IAEA and NNEA RIA kits, and the results were compared.

3. RESULTS

3.1. Survey on reproduction of cattle bred by AI

3.1.1. Training activities

During the initial stages of the project, 12 training activities were conducted for veterinarians, AI technicians, extension workers and farmers, to introduce the application of the AIDA recording system and the RIA progesterone technique. Approximately 400 participants attended these activities, amounting to around 800 man/days of training.

Training workshops conducted during the latter stages of the project were aimed at consolidating the AIDA and RIA techniques for monitoring and improving AI services and for the diagnosis of sub-fertility. The activities included 5 for farmers (68 participants), 3 for veterinarians (28 participants), 3 for AI technicians and extension workers (29 participants) and 3 for scientists and technical staff (30 participants).

3.1.2. West Sumatra

Results from the baseline survey on reproductive performance of locally adapted beef cattle bred by AI at two locations in West Sumatra are given in Table III. An analysis of the reproductive performance in relation to various factors that were recorded during the survey is given in Table IV.

TABLE III. REPRODUCTIVE PERFORMANCE OF LOCALLY ADAPTED BEEF CATTLE IN WEST SUMATRA

Parameter	Location 1 (n=49)	Location 2 (n=49)	Overall (n=98)
Calving to first AI (days)	150.0 ± 112.3	256.4 ± 99.8[a]	203.5 ± 118.3
Calving to conception (days)	173.6 ± 139.0	237.1 ± 66.8[b]	208.4 ± 108.5
Services/Conception	3.6	2.9	3.2
Conception rate (%)	28.0	34.7	31.3

[a,b]Number of animals used to calculate conception interval differed from that for interval to first AI

3.1.3. South Sulawesi

The effect of feed supplementation with UMMB on the reproductive performance of dual-purpose cattle (beef and draught) of the Bali breed subjected to AI in South Sulawesi is shown in Table V.

The cattle given supplementary feeding had shorter mean calving to conception interval and higher mean conception rate (CR) than the controls.

TABLE IV. EFFECTS OF DIFFERENT MANAGEMENT SYSTEMS AND AI TECHNICIANS ON REPRODUCTIVE PERFORMANCE OF BEEF CATTLE IN WEST SUMATRA

Parameter	Interval (d) from calving to		Conception	Services/
	First AI[a]	Conception[b]	Rate (%)	Conception
Housing System:				
Corral/paddock	229.5 ± 110.2	249.4 ± 70.4	28.8	3.5
Loose barn	138.9 ± 112.9	95.4 ± 55.4	36.8	2.7
Tie stall	188.4 ± 125.7	220.6 ± 150.6	33.3	3.0
Feeding system:				
Grazing + concent.	256.9 ± 103.4	199.0 ± 36.8	28.6	3.5
Grazing + roughage	143.3 ± 112.9	198.1 ± 161.2	36.8	2.7
Grazing only	214.5 ± 117.3	212.5 ± 96.3	30.6	3.3
AI technician:				
Inseminator 1	–	–	31.0	3.2
Inseminator 2	–	–	34.0	2.9
Employer of AI technician:				
Government	–	–	32.2	3.1
Private	–	–	16.7	6.0

[a,b]Numbers of animals used to calculate conception interval differed from that for interval to first service

TABLE V. EFFECTS OF FEED SUPPLEMENTATION WITH UMMB ON THE REPRODUCTIVE PERFROMANCE OF BEEF CATTLE IN SOUTH SULAWESI

Parameter	Control (no UMMB; n = 59)	Treatment (with UMMB; n = 59)
Calving to first service[a] (d)	150.6 ± 66.3	102.3 ± 36.5
Calving to conception[b] (d)	175.9 ± 56.3	104.0 ± 36.6
No of locations (villages)	3	2
No of farmers	45	27
CR (%)	27.1	49.2

[a,b]Numbers of animals used to calculate conception interval differed from that for interval to first service

3.1.4. West Java

The results from measurement of progesterone in the three milk samples collected on the day of AI (day 0) and on days 10–12 and 21–24 after AI are shown in Tables VI, VII and VIII. Table VI contains the interpretation of results based on the first sample only, while Table VII contains the interpretation based on the first two samples. Table VIII has the overall interpretation, based on results from all three samples of milk together with the findings from rectal palpation.

The reproductive performance of dairy cattle in Garut district of West Java before and after undertaking interventions, which comprised feed supplementation with UMMB and training of farmers on improved feeding and reproductive management, is shown in Table IX.

TABLE VI. DIAGNOSIS OF REPRODUCTIVE STATUS OF CATTLE BASED ON PROGESTERONE CONCENTRATION IN ONE SAMPLE OF MILK COLLECTED AT THE TIME OF AI (DAY 0)

Progesterone concentration	No. of samples	Frequency (%)	Interpretation
Low (<1 nmol/L)	97	65.5	AI done at a time other than luteal phase (probably at appropriate time)
High (>3 nmol/L)	34	23.0	AI done during luteal phase (clearly at inappropriate time)
Intermediate (1.1–2.9 nmol/L)	17	11.5	Further information is necessary for interpretation. AI may have been done too early or too late relative to oestrus
Total samples	148		

TABLE VII. DIAGNOSIS OF REPRODUCTIVE STATUS OF CATTLE BASED ON PROGESTERONE CONCENTRATION IN TWO SAMPLES OF MILK, COLLECTED AT THE TIME OF AI (DAY 0) AND 10–12 DAYS LATER

Progesterone		No. of samples	Frequency (%)	Interpretation
Day 0	Day 10–12			
Low	High	45	31.5	AI done at an ovulatory oestrus
Low	Low	38	26.6	Anoestrus, anovulation or short luteal phase
High	Low	3	2.1	AI done during luteal phase and a possibility of next heat occurring after 7–14 days
High	High	13	9.1	AI done on pregnant animal or with luteal cyst
*	*	44	30.8	Other clinical data is required for proper interpretation
Total samples		143		

*At least one of the samples showed an intermediate value (1.1–2.9 nmol/L)

TABLE VIII. DIAGNOSIS OF REPRODUCTIVE STATUS OF CATTLE BASED ON PROGESTERONE CONCENTRATION IN THREE SAMPLES OF MILK, COLLECTED AT THE TIME OF AI (DAY 0), 10–12 AND 22–24 DAYS LATER, AND RECTAL PALPATION FOR PREGNANCY DIAGNOSIS

Progesterone			Pregnancy diagnosis	No. of samples	Frequency (%)	Interpretation
Day 0	Day 10–12	Day 22–24				
Low	High	High	Pregnant	35	33.7	Pregnant (successful insemination)
High	High	High	Pregnant	9	8.7	AI done on pregnant animal
Low	Low	Low	Non Pregnant	26	25.0	Anoestrus or anovulation
*	*	*	Pregnant or Non-pregnant	34	32.7	Other clinical data is required for proper interpretation.
Total samples				104		

*At least one of the samples showed an intermediate value (1.1–2.9 nmol/L)

TABLE IX. REPRODUCTIVE PERFORMANCE OF DAIRY CATTLE FROM TWO LOCATIONS IN GARUT DISTRICT, BEFORE AND AFTER SUPPLEMENTARY FEEDING AND TRAINING OF FARMERS

Parameter	Overall		Location 1		Location 2	
	Before	After	Before	After	Before	After
Calving to first AI (days)	121.3 ± 78.2 (n = 137)	112.1 ± 80.9 (n = 161)	112.6 ± 68.1 (n = 107)	80.4 ± 63.3 (n = 120)	152.0 ± 103.2 (n = 30)	143.8 ± 98.6 (n = 41)
Calving to conception (days)	135.8 ± 68.2	135.1 ± 77.6	122.6 ± 32.4	102.1 ± 50.0	−	168.1 ± 105.2
Services per conception	3.0	2.7	3.3	2.9	2.3	1.6
Conception rate (%)	33.8	37.3	30.2	34.2	44.4	61.0

3.2. Application of progesterone RIA for early diagnosis of reproductive disorders

3.2.1. West and Central Java

During the baseline survey, 20 dairy cows were identified as having reproductive problems. There were no individual records and only the immediate past history on postpartum events was recalled by the farmers. Investigations using progesterone measurement and clinical examination of the reproductive organs revealed a range of disorders, as described below.

Lack of observed oestrus was a common complaint in many of the problem cows. Some of these cows had basal progesterone concentration over long periods, as shown in Figure 1, indicating a complete lack of ovarian cyclicity. The diagnosis was true anoestrus and the recommendations to the farmers included improvement of feeding, observation for oestrus and, in old cows with poor body condition, culling.

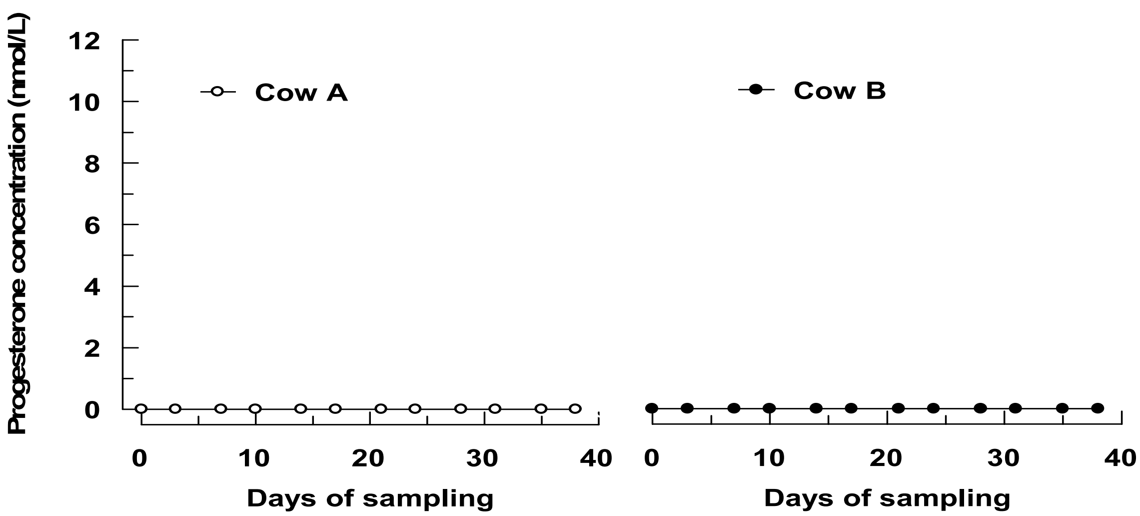

FIG. 1. Milk progesterone profile from two dairy cows with true anoestrus.

In some cows with a history of anoestrus progesterone profiles revealed that ovarian activity was in fact occurring, as shown in Figure 2, leading to a diagnosis of missed oestrus. The day on which the previous oestrus occurred was deduced from the progesterone profile and the likely date of next oestrus was calculated. The farmers were given instructions on ways to improve heat detection and on obtaining AI services at the appropriate time. The two cows shown in Figure 2 were subsequently inseminated and confirmed to be pregnant.

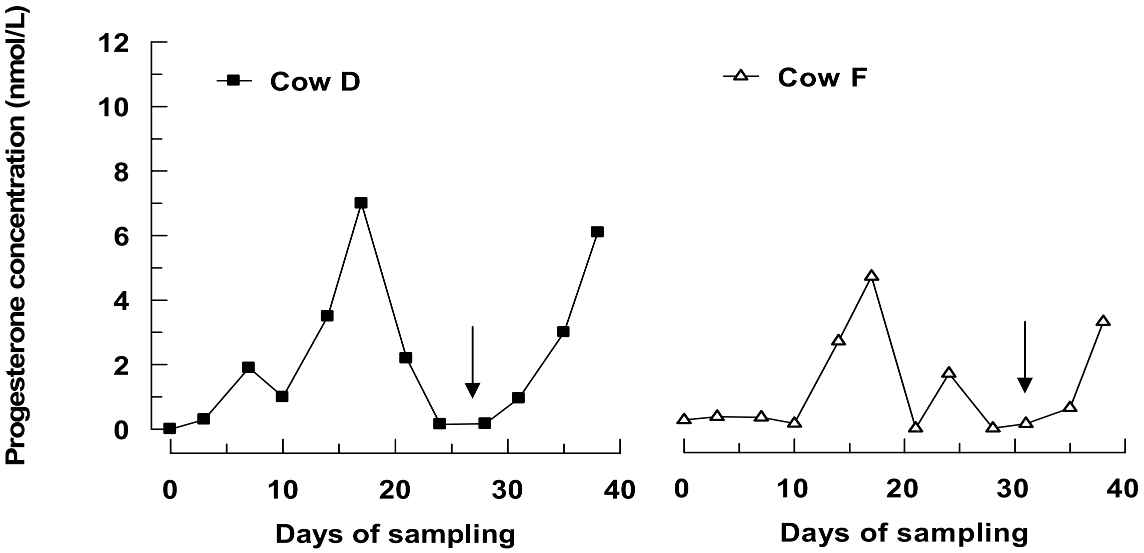

FIG. 2. Milk progesterone profile in two dairy cows with missed oestrus (arrows indicate the likely day when the previous oestrus occurred).

Milk progesterone concentrations in one cow showed a cyclic pattern with an interval of 28 days between successive periods of basal progesterone (Figure 3). The diagnosis was prolonged luteal phase, which could be due to a persistent CL or late embryo death. The likely day of occurrence of the next oestrus was predicted and the farmer was given advice on heat detection and timely AI. The cow was subsequently confirmed to have been inseminated and conceived.

Progesterone profiles in two cows that were recipients of embryo transfers are shown in Figure 4. They had received the transferred embryos 45 and 46 days before commencement of sampling and were diagnosed as having failed to conceive. The progesterone profiles indicated that they were cyclic and it was inferred that the embryos had died sometime before sampling commenced.

Figure 5 shows the milk progesterone profile of two cows that appear to have been inseminated during the early luteal phase. The sampling commenced one day after AI and although the profile suggests that the ensuing cycle may have been longer than 21 days, it is not possible to confirm this because the sampling frequency was only once in 10 days.

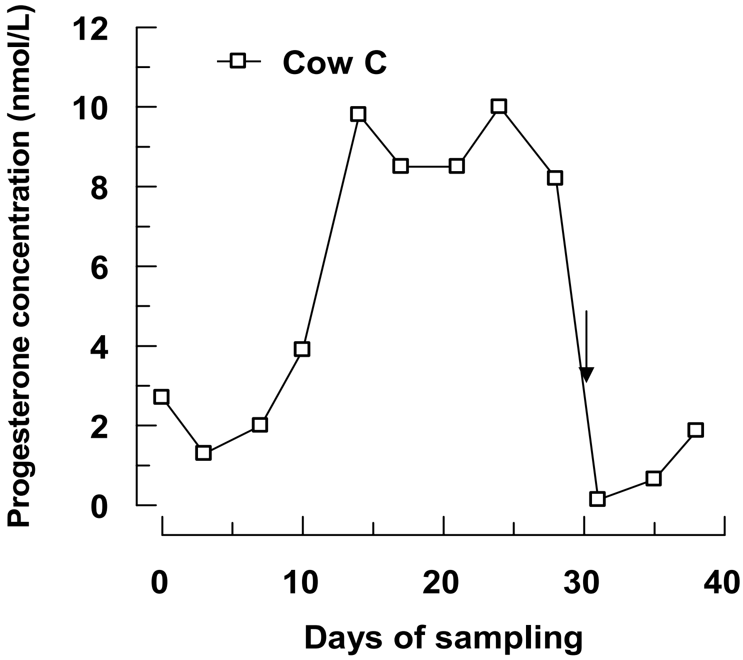

FIG. 3. Milk progesterone profile of a cow suspected to have a prolonged oestrus cycle of 28 days.

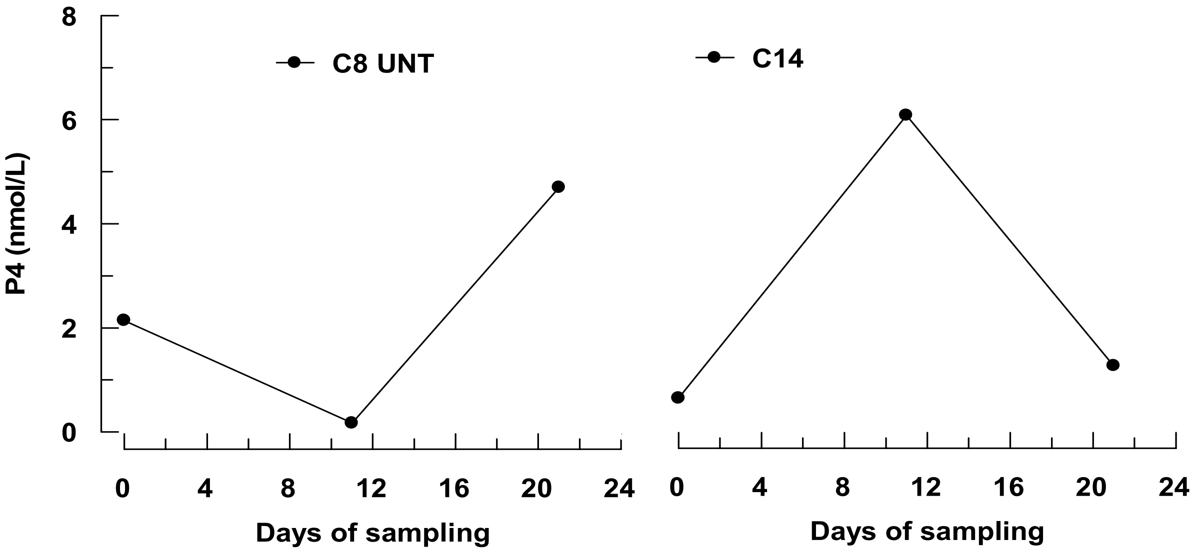

FIG. 4. Milk progesterone profiles of two cows that received embryo transfers at days 45 and 46 before commencement of sampling.

The progesterone profiles shown in Figure 6 indicate that these cows were also inseminated during the luteal phase. Within the limitations of the sampling frequency it can be suspected that the profile shown on the left indicates AI during the mid-luteal phase, while that on the right indicates AI during the late luteal phase.

The progesterone profiles shown in Figure 7 indicate that these cows were either pregnant at the time of insemination, or had persistent CLs or luteal cysts.

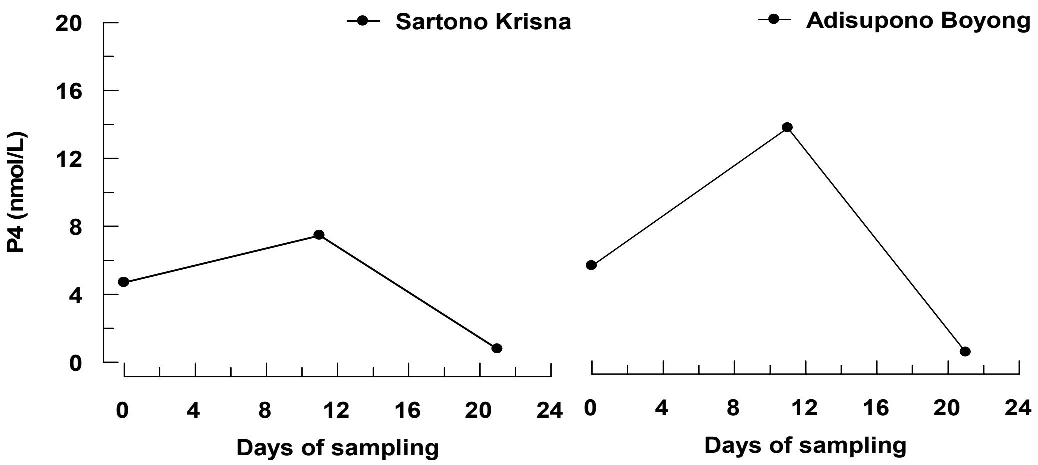

FIG. 5. Milk progesterone profiles of two cows that appear to have been inseminated during the early luteal phase (sampling commenced one day after AI).

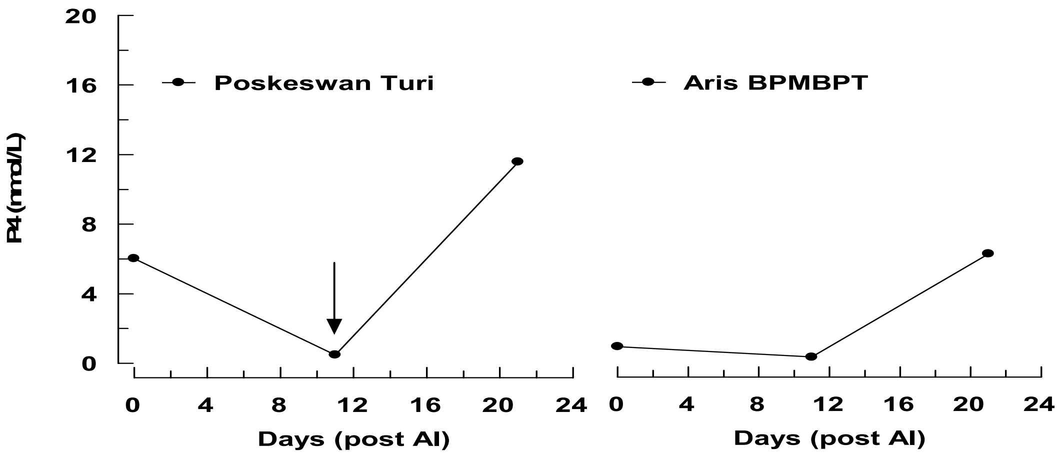

FIG. 6. Milk progesterone profiles of two cows that appear to have been inseminated during the mid (left) and late (right) luteal phase.

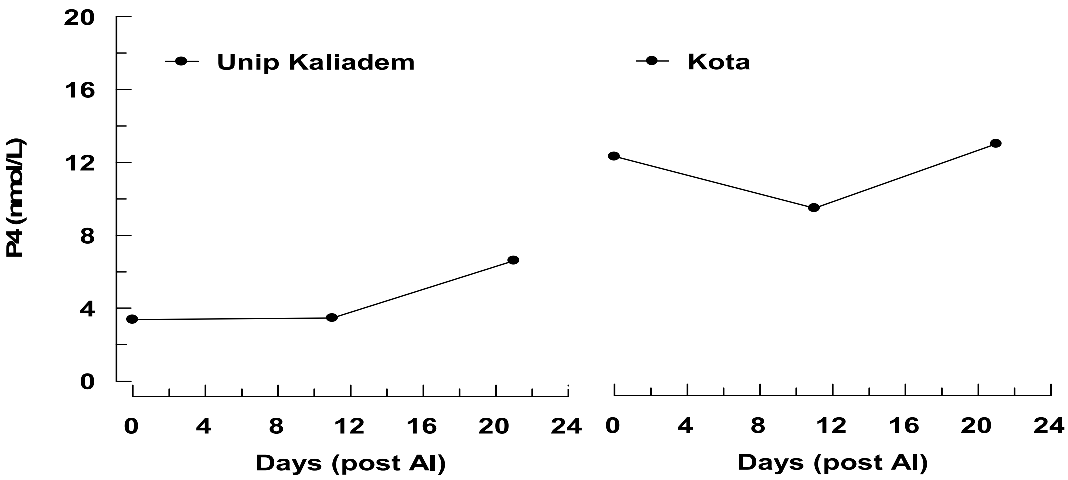

FIG. 7. Milk progesterone profiles of two cows that appear to have been inseminated during pregnancy or had persistent corpora lutea or luteal cysts.

In the above cases where AI had been done at inappropriate times, the farmers were advised on methods for improving heat detection and given instructions on keeping simple records so as to avoid such errors in future. The training conducted for AI technicians also emphasized the importance of assessing the status of animals before performing AI.

3.2.2. Lombok Island, West Nusa Tenggara

Table X shows the results from progesterone measurement and subsequent rectal palpation on Brahman cattle at two locations in Lombok Island.

TABLE X. REPRODUCTIVE STATUS OF BRAHMAN CATTLE DETERMINED FROM PROGESTERONE MEASUREMENT AND RECTAL PALPATION

Location	No. of animals	Cyclic	Acyclic	Persistent CL[a]	Follicular cyst	Pregnant
West Lombok	18	5	6	5	–	2
East Lombok	50	30	7	10	1	2

[a]Corpus luteum

3.2.3. Economic evaluation

The economic evaluation done on the application of progesterone measurement by RIA using the NNEA kit produced locally showed that the cost of collecting and analyzing one sample was INS Rp 37 500 (US $1.00 = INS Rp 9,300). Therefore, the total cost of using the three-sample regime would be INS Rp 112 500 per animal.

The current price of one AI using local or imported semen varies from INS Rp 20 000 to 40 000 with an average of INS Rp 30 000. Thus, cases with sub-fertility requiring three services for conceptioncost farmers an average of Rp 90 000. Additional costs in maintaining and feeding such animals for 42–61 days until conception costs on average INS Rp 210 000, with the assumption that daily feeding cost is INS Rp 4000 per animal.

Therefore, the economic advantage to the farmer in utilizing the progesterone RIA technique for early detection of AI failure and sub-fertility, assuming that using the test leads to conception, is approximately INS Rp 187 500 (i.e. Rp 90 000 + 210 000 – 112 ,500), which is equivalent to US$ 20.10 per animal.

3.3. Development and production of progesterone RIA kits in Indonesia

The immunization of three rabbits with progesterone-11α-hemisuccinate resulted in all the animals producing anti-progesterone antibody. The anti-progesterone serum was tested at various dilutions for binding against ^{125}I-progesterone tracer that was supplied with the IAEA kit and the results are shown in Figure 8. The binding at 1:100, 1:1000 and 1:12 500 were 21%, 35% and 30%, respectively.

The results from the iodination of progesterone using the Chloramin-T method and separation of free and labeled progesterone using column chromatography are shown in Figure 9. The tests done on the quality and stability of the ^{125}I-progesterone tracer produced are shown in the Table XI.

The results indicate that the purified tracer had fully acceptable characteristics and was stable for up to 12 weeks.

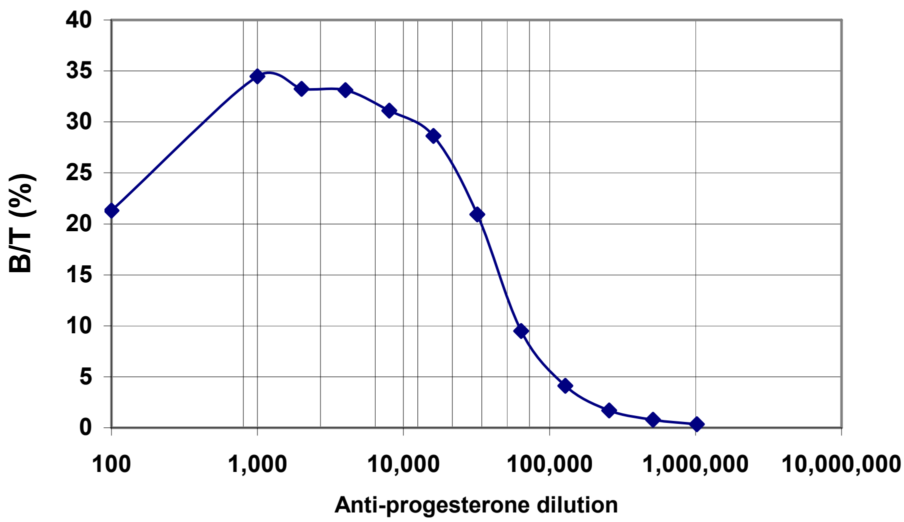

FIG. 8. Percentage binding relative to total counts (B/T%) for the anti-progesterone serum against progesterone tracer.

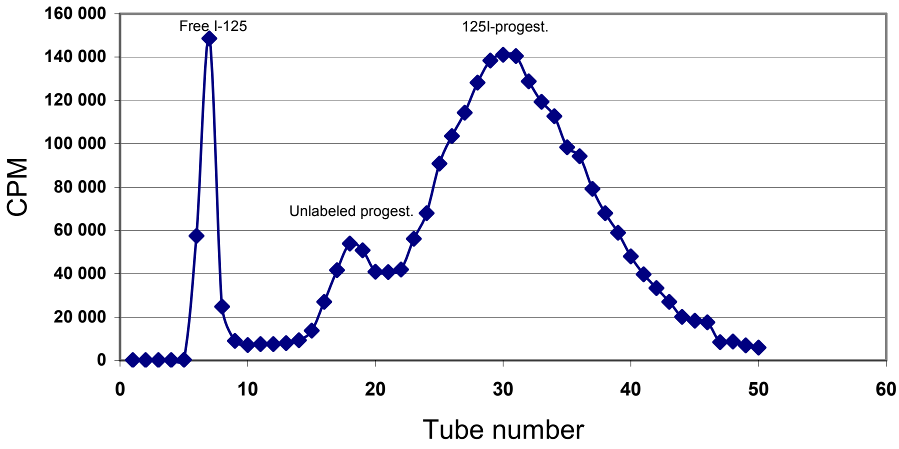

FIG. 9. Iodination of progesterone using the Chloramin-T method and separation of free and labeled progesterone using column chromatography.

TABLE XI. QUALITY AND STABILITY OF THE ^{125}I-PROGESTERONE TRACER PRODUCED BY THE NNEA

Parameter	Weeks after production					
	0	1	2	7	12	13
Labeling efficiency (%)	57.2	28.8	75.7	92.6	92.8	98.5
Radiochemical purity (%)	–	92.0	93.3	97.5	90.0	87.4
B/T, max binding (%)	–	63.6	57.2	64.9	48.3	52.0
Non-specific binding (%)	0.3	0.8	0.4	0.7	0.3	0.7
Specific activity (µCi/µg)	114.2	55.6	151.3	390.7	426.9	473.0

The progesterone standards prepared in skim milk were used in the RIA and statistical calculations were done using the homogenous subsets test of Duncan. The standards 0, 2.5, 5.0, 10.0, and 20.0 nmol/L had coefficients of variation (CVs) of 0.3%, 2.3%, 6.0%, 11.2% and 19.1%, respectively.

Although all above results are within acceptable limits, the results from assaying control samples in routine RIA using the NNEA kit (with antiserum, tracer and standards prepared locally) gave an intra-assay CV of 24.7% and an inter-assay CV of 30.8%. Furthermore, a comparison between the results obtained using the IAEA and NNEA kits showed a variation of 39.2%. These results are above the acceptable limits and indicate the need for further investigations to improve the performance and stability of the NNEA kit. The most likely problem is in the quality and stability of the standards prepared in skim milk and could be due to the commercial product used. Further work is in progress to address this.

4. DISCUSSION

4.1. Survey on reproduction of cattle bred by AI

The farms in West Sumatra, South Sulawesi, Lombok Island and Garut in West Java represent the typical smallholding mixed crop-livestock farming systems in Indonesia. The first three locations have predominantly beef cattle, with similarities in housing, feeding and heat detection systems. The fourth location has mainly dairy cattle. In all four systems the farmers do not have records on their animals and do not practice a regular systematic culling programme on the basis of production or age. The investigations and interventions undertaken during this project are still continuing in all locations.

The average interval from calving to first AI was different at the four locations, ranging from 121.3 ± 78.2 days in West Java to 203.5 ± 118.3 in West Sumatra. This delay of first service after calving, particularly in beef cattle, appears to be due to prolonged postpartum anoestrus. This is most likely a result of poor nutrition and suckling management, the latter arising from the lack of restriction on suckling by the calves in beef cattle systems. On the other hand, prolonged interval to AI in dairy cattle is often due to nutrition and mating management, the latter arising from the tendency by many farmers to delay service, in order to have a longer lactation period. Additional investigations are needed to determine whether the prolonged postpartum anoestrus is primarily due to management or nutritional factors. The observation that feeding interventions using UMMB had an effect on shortening the interval to fist service indicates that nutrition may be a limiting factor in many farms. Prolonged postpartum anoestrus and repeat breeding were obviously major problems in crossbred beef animals of West Nusa Tenggara, where most farmers tended to cull these animals and replace them with native breeds.

The present study showed that CR in beef and dairy cattle subjected to AI ranged from 27–35% at the different locations. A major reason for this low CR appears to be poor heat detection and inappropriate timing of AI. Progesterone concentration on the day of AI was high in 23% of animals, indicating that they were not in a suitable stage for AI. Based on progesterone levels in the first two samples (days 0 and 10–12) it was found that only 31.5% of animals presented to AI appeared to have had a normal ovulatory oestrus. Furthermore, results from all three samples revealed that 25% of the animals were non-cyclic or anovulatory, while 8.7% were pregnant at the time of AI. Therefore, it is clear that failure of AI services is largely due to improper timing of AI, especially in dairy farms. This is most probably because firstly, farmers rely on secondary signs for heat detection and secondly, the inseminators do not verify the heat status of animals before performing AI.

Previous findings [7] have shown that the timing of insemination in relation to first detection of heat is critical for achieving high conception rates. Therefore, interventions undertaken included improving the knowledge and skills of AI technicians and farmers through training and education activities. The results from South Sulawesi and West Java indicate that such interventions resulted in a reduction of the number of services needed to achieve a conception and an increase in the CR of both beef and dairy cattle.

It is also known that factors related to the transport, storage, handling and thawing of semen in the field are very important determinants of conception rates to AI [7]. The continuing studies under this project are now monitoring these factors.

4.2. Application of progesterone RIA for early diagnosis of reproductive disorders

The animals included in this study were those that the farmers reported as having reproductive problems. As shown in previous studies [8] the use of progesterone measurement combined with the breeding history obtained from the farmer and clinical examination by a veterinarian provide a comprehensive picture of the reproductive status of each animal and the underlying hormonal problem, if any.

A common problem reported by farmers was absence of heat signs over a long period after calving. The results from progesterone measurement and clinical examination showed that these animals were having a range of problems, including true anoestrus, sub-oestrus or missed oestrus, persistent CL and luteal cysts. Arriving at an accurate diagnosis permitted the appropriate advice or treatment to be provided for overcoming the problems. In some cases the animals had reached their sixth or seventh parity and were therefore in a phase of declining reproductive efficiency [9, 10]. Culling was recommended for such cows.

Some of the cows reported to be anoestrous were found to have resumed ovarian activity. In such cases the likely timing of the next oestrus period was deduced from the progesterone profile, and advice was given to farmers on heat detection and timing of AI. Subsequent rectal palpation revealed successful conceptions in a high proportion of these cows.

The use of BCS to assess nutritional status was found to be useful for providing advice to the farmers on appropriate feeding of their cows in relation to the stage of production. Previous studies have shown an association between BCS and postpartum anoestrus, which can be overcome by nutritional management [11]. Furthermore, it has been stated that appropriate nutritional management of the prepartum dairy cow during the transition phase to reduce the incidence of calving related disorders (i.e. milk fever, dystocia, retained fetal membranes, ketosis and metritis), which alone or collectively reduce reproductive success, is an integral component of a reproductive herd health program. However, in most dairy herds, attention is given to nutrition and management of the transition cow only after problems occur. Therefore, to prevent calving related disorders from occurring and resulting in major economic losses, periodic evaluation of prepartum and postpartum management of transition cows is recommended.

Overall, the application of progesterone RIA in this study revealed that the main causes for reproductive failure or sub-fertility were postpartum anoestrus, missed oestrus, repeat breeding and prolonged oestrous cycles. Most of these failures were related to the management system as a whole, which comprises nutritional, reproductive and health management [2, 12]. Thus an integrated approach is necessary for improving the productivity of these smallholder beef and dairy farming systems. However, difficulties were encountered in introducing new methods involving recording and improvements to the overall management system. These problems arise due to traditional culture and lack of understanding of dairy herd management by the farmers. However, by demonstrating the AIDA recording system and utilizing progesterone RIA, improvements were possible in the management of beef and dairy cattle by farmers and the quality of services provided by AI technicians and extension workers.

The main outcomes from the project at the various study locations can be summarized as: (a) improvement of existing recording systems, with more uniformity to conform to the AIDA protocol; (b) identification of main limitations and designing of specific interventions to improve production efficiency; (c) wider understanding on the applicability of progesterone

RIA for early diagnosis of sub-fertility, resulting in a demand for establishing decentralized mini-laboratories; and (d) reduction in the number of AI services required per conception.

4.3. Development and production of progesterone RIA kits in Indonesia

This study has demonstrated that anti-progesterone serum and ^{125}I-progesterone tracer for use in RIA can be successfully produced in Indonesia. The tracer had good stability for up to 12 weeks, with a constant specific activity throughout the period of observation. The production of standards in skim milk needs improvement and the RIA requires further work for optimization and validation. However, the prospects for producing progesterone RIA kits within Indonesia to meet the future needs of the country are good.

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REPRODUCTIVE STATUS FOLLOWING ARTIFICIAL INSEMINATION AND FACTORS AFFECTING CONCEPTION RATE IN DAIRY COWS IN SMALLHOLDER PRODUCTION SYSTEMS

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Abstract

REPRODUCTIVE STATUS FOLLOWING ARTIFICIAL INSEMINATION AND FACTORS AFFECTING CONCEPTION RATE IN DAIRY COWS IN SMALLHOLDER PRODUCTION SYSTEMS

A survey was conducted to evaluate the reproductive status following artificial insemination (AI) and factors affecting conception rate (CR) in dairy cows under the smallholder production system, using the concentration of progesterone (P4) in milk samples taken on the day of AI (Sample 1), day 10–12 after AI (Sample 2) and day 22–24 after AI (Sample 3). The survey involved 115 cows in 33 farms. A follow-up study was carried out on four farms with interventions to improve record keeping, feed supplementation, heat detection and timely pregnancy diagnosis. Based on Sample 1 (n = 115), 93% of the cows had low P4 and were likely to have been in or close to oestrus at AI. Based on Samples 1 and 2 (n = 107), 85% of the cows had ovulatory oestrus. Based on all three samples (n = 59), 54.2% of the cows appeared to have conceived, 18.6% had either non-fertilization or early embryonic mortality and 18.6% had late embryonic mortality, luteal cyst or a persistent corpus luteum. The incidence of AI on pregnant animals was 1.7% and on those in doubtful reproductive status was 6.8%. The overall CR was 35.5% from 121 inseminations done on 115 cows. Mean intervals from calving to first AI (n = 77) and to conception (n = 43) were 90.7 and 113.6 days, respectively.

The effects of level of milk production, lactation state and site of semen deposition on CR were significant ($p < 0.05$), as CR was higher in cows with high milk yield, those which were not suckled and those in which the semen was deposited in the body of the uterus. Other factors were not significant ($p > 0.05$) but CR tended to be lower in first parity cows and in cows with excessive body condition. The CR was also lower in farms that practice AI only in the afternoon, in farms where relatively less time was spent on dairy activities and in those farms practicing grazing and supplementation with concentrate only, as compared to those providing additional roughage supplementation. CR tended to be higher when AI was carried out by technicians with longer formal training.

The survey showed that there was a high occurrence of ovulatory oestrus in cows under the smallholder production system but the CR obtained was low. This result can be attributed to factors related to the cow, nutrition, management and AI procedures. The interventions done on four farms resulted in shorter intervals from calving to first AI and conception, lower number of services per conception and higher CR, when compared with findings from the survey.

1. INTRODUCTION

The use of artificial insemination (AI) in Malaysia as a breeding technique has been declining for the past several years. The total number of AI carried out in 1993 was 30 638 and decreased to 17 527 in 2000 [1]. Low conception rate (CR) was one of the main reasons for this decline, while difficulties in obtaining the service in a timely manner due to logistic and communication obstacles also contributed to it. As a result, many dairy farmers resorted to the use of bulls to breed their animals.

It is recognized that low CR can be caused by several factors and their interactions, including those related to the cow, management of the animal, AI services, semen quality and bull fertility. Apart from these, reproductive events such as occurrence of oestrus, ovulation, conception and embryonic mortality will also influence CR. The occurrence of these reproductive events should be assessed so that the extent of their influence on fertility can be quantified and appropriate measures adopted to improve CR. The relative contribution of the factors mentioned above should also be determined so that a holistic approach can be adopted to improve the fertility of cows on dairy farms.

The objective of the study was to assess the occurrence of reproductive events (oestrus, ovulation, conception, embryonic mortality and anoestrus) following AI and to evaluate the extent of influence of factors affecting CR in dairy cows under smallholder dairy production systems. Based on the findings of the survey, specific interventions were undertaken in selected farms to demonstrate to farmers that improvement in reproductive performance can be achieved through sound management practices.

2. MATERIALS AND METHODS

2.1. Survey

A survey was conducted on 33 small dairy farms involving 115 Sahiwal-Friesian crossbred cows aged between four and eight years. The farms were located in five regions in the southern part of peninsular Malaysia. Survey forms as described in the user manual for the Artificial Insemination Database Application for Asia (AIDA Asia) [2] provided by the Animal Production and Health Section of IAEA were used in the survey. Information on the farm, AI technician, semen used, management practices, cow inseminated and AI related services were recorded by AI technicians on the day of AI.

Milk samples from cows bred by AI were collected on the day of AI (Sample 1), on day 10–12 after AI (Sample 2) and on day 22–24 after AI (Sample 3) using 10 mL tubes containing one sodium azide tablet as a preservative. The milk samples were chilled at 4°C and subsequently centrifuged at room temperature (2300 g, 15 min) to remove the fat. The fat-free milk was stored at –21°C until analysed for progesterone (P4) concentration. The P4 concentrations in the skim milk were determined by solid-phase radioimmunoassay (RIA), using kits supplied by the Animal Production and Health Section of the IAEA. The procedure for the assay was as described in the manual provided by the IAEA (Progesterone RIA ‘Pre-coated Tube' Method, Assay Protocol Version 3.1, 1996). Antibody pre-coated tubes were used together with ^{125}I labelled progesterone as the isotope tracer and the radioactivity of the tracer in the standard, quality control and unknown samples were measured in a gamma counter (Series E 5005 Cobra; Packard Instrument Company). The intra- and inter-assay coefficients of variation were 3.7% and 10.7%, respectively.

Body condition score (BCS) of cows was assessed by visual inspection on the day of AI and a grading scale of 1 to 6 (1 = very thin; 2 = thin; 3 = satisfactory; 4 = good; 5 = very good; 6 = fat) was used. Pregnancy diagnosis (PD) through rectal palpation was carried out two to four months after AI.

Cows were categorized according to their reproductive status based on the P4 values using the guidelines described in the AIDA Asia manual [2]. Accordingly, P4 concentrations from undetectable to <1 nmol/L, 1–3 nmol/L and >3 nmol/L were designated as low, intermediate and high, respectively. Sample 1 was used to verify whether the animal was likely to be in oestrus or the luteal phase of the cycle (high P4 values indicating incorrect timing). Samples 1 and 2 were used to verify whether the cow was cycling and had ovulated and Samples 1, 2 and 3, together with results of PD, were used to verify whether the animal had conceived, had lost the embryo, had a persistent corpus luteum, had been inseminated while pregnant or had been inseminated when acyclic. Overall, one milk sample was taken from 115 cows, two milk samples from 107 cows and three milk samples from 59 cows.

All survey data, including the P4 concentration in milk samples and the result of PD, were entered into AIDA Asia and the application's reports facility was used to generate summaries of results. The reproductive status of the cows was evaluated in terms of percentages of occurrence of the reproductive events mentioned above and the Chi-square test was performed on the enumeration data to determine the statistical significance of effects of factors influencing the CR.

2.2. Interventions

Four small dairy farms (less than 10 cows) that had a problem of repeat breeding (requiring three or more services to get a cow pregnant) were selected. All the cows in the selected farms were Sahiwal-Friesian crossbreds aged from five to seven years. The cows grazed on native pastures from 11:00 to 16:00 hr and were confined in a barn at night. Feed supplements comprising soybean waste, tapioca chips, palm kernel cake, rice bran or sago pulp meal were given as single ingredients when available. The farmers did not maintain proper record keeping and most of the time relied on their memory to recall some of the reproductive events occurring among their animals. The following interventions were adopted on the four farms: (a) record keeping that must include reproduction data (date of calving, date of AI); (b) feed supplementation (oil palm frond-based diet in the form of cubes, 90% DM, 14% CP, 9.1 MJoule of ME/kg) given at 1% body weight from 4–6 weeks prior to calving to 60 days after calving; (c) routine oestrus detection beginning 30 days after calving and 18–30 days after AI; (d) AI to be done by an experienced technician, following the AM/PM rule on the timing of AI in relation to oestrus detected; (e) thawing of semen in warm water and deposition in the uterus; and (f) pregnancy diagnosis to be done by rectal palpation 60 days after AI.

3. RESULTS

3.1. Reproductive status based on milk progesterone concentrations

Based on the P4 concentration in the first milk sample, 93% of the cows inseminated were likely to be in or close to oestrus, 2.6% were probably not in oestrus and 4.3% were clearly not in oestrus (Table I). Based on the first two samples, the occurrence of ovulatory oestrus was 85%, while cows that were anovulatory or had short luteal phases accounted for 3.7% (Table II). Incidence of cows with luteal cysts was 1.9% and those with unconfirmed reproductive status (requiring further clinical data) accounted for 9.3%.

TABLE I. REPRODUCTIVE STATUS OF COWS BASED ON P4 LEVEL IN MILK SAMPLES (N = 115) TAKEN ON THE DAY OF AI

Milk P4 concentration	Reproductive status	No. of animals	Percentage
Low (<1 nmol/L)	Probably in oestrus	107	93
Intermediate (1–3 nmol/L)	Probably not in oestrus	3	2.6
High (>3 nmol/L)	Clearly not in oestrus	5	4.3

TABLE II. REPRODUCTIVE STATUS OF COWS BASED ON P4 LEVEL IN MILK SAMPLES (N = 107) TAKEN ON THE DAY OF AI (DAY 0) AND ON DAY 10–12 AFTER AI

Milk P4 concentration		Reproductive status	No. of animals	Percentage
Day 0	Day 10–12			
Low	High	Ovulatory oestrus	91	85
Low	Low	Anoestrus, anovulation or short luteal phase	4	3.7
High	High	Luteal cyst	2	1.9
*	*	Other clinical data is required for proper interpretation	10	9.3

*At least one of the samples showed an intermediate value (1–3 nmol/L)

Based on the P4 concentration in all three milk samples, 54.2% of the inseminated cows had probably conceived, 18.6% had either fertilization failure or early embryonic mortality and another 18.6% had either late embryonic mortality or persistent corpora lutea (Table III), corresponding to a reproductive wastage of 37.2%. Incidence of AI on pregnant cows was 1.7% and of cows with an unconfirmed reproductive status (requiring other clinical data for proper interpretation) was 6.8%.

Thirty-three of 44 cows with high progesterone values on day 22–24 after AI were confirmed pregnant after pregnancy diagnosis, giving a 75% accuracy of early pregnancy diagnosis, while all 11 cows with low progesterone values on day 22–24 after AI were confirmed not pregnant, giving a 100% accuracy of early non-pregnancy diagnosis by using milk P4 RIA.

Overall, 121 AI services were done on 115 cows and the CR, services per conception and intervals from calving to first AI and CR are shown in Table IV.

3.2. Effect of factors related to cow on conception rate

The effect of suckling was significant (P <0.05); cows subjected to milking without suckling had higher CR than did cows subjected to milking and suckling (Table V). The effect of level of milk production on CR was highly significant (P <0.01) in that high producing cows had higher CR than did low producing cows.

Effects of other factors related to cows on CR were not significant. However, CR tended to be higher in multiparous than in primiparous cows and in cows that had obvious signs of heat such as standing heat, mounting others and marked uterine tone. CR also tended to be higher in cows with BCS of 4 compared to that in cows with BCS of 5 to 6.

TABLE III. REPRODUCTIVE STATUS OF COWS BASED ON P4 LEVEL IN MILK SAMPLES (N = 59) TAKEN ON DAY 0, DAY 10–12 AND DAY 22–24 AFTER AI FOLLOWED BY MANUAL PREGNANCY DIAGNOSIS

MILK P4 CONCENTRATION			MANUAL PALPATION	REPRODUCTIVE STATUS	%
Day 0	Day 10–12	Day 22–24			
Low	High	High	Positive	Pregnant	54.2
Low	High	Low	Negative	Non-fertilization, early embryonic mortality, post AI anoestrus	18.6
Low	High	High	Negative	Late embryonic mortality, luteal cyst, persistent CL	18.6
High	High	High	Positive	AI on pregnant animal	1.7
*	*	*	Positive/ Negative	Other clinical data is required for proper interpretation	6.8

*At least one of the samples showed an intermediate value (1–3 nmol/L)

TABLE IV. REPRODUCTIVE PERFORMANCE OF COWS FOLLOWING AI

	No. of observations	Mean ± SD
Conception rate (%)	115	35.5
Services per conception	121	2.8
Calving to first AI (days)	77	90.7 ± 35.2
Calving to conception (days)	43	113.6 ± 63.7

3.3. Effect of factors related to farm on conception rate

There was no significant effect of factors related to the farm on CR (Table VI). However, CR tended to be higher in cows under a feeding system consisting of grazing + concentrate + roughage (cut grass) as compared to grazing + concentrate, in cows bred in the AM (antemeridiem/before noon) than PM (postmeridiem/after noon) and in cows bred 12 hours after heat signs were observed. Farmers spending more time in dairy activities also tended to improve the CR. Excluding the Kluang region due to very few numbers of observations, dairy cows in the Jasin region had higher CR than in the other three regions.

3.4. Effect of factors related to AI technician and bull

The effect of site of semen deposition on CR was highly significant (P <0.01). Depositing semen in the uterus resulted in higher CR than depositing semen in the cervix. There was no significant effect of AI technicians, method of thawing and length of formal training on CR (Table VII). However, more conceptions were obtained when semen was thawed in warm water as opposed to cold water and CR tended to be higher when the AI was done by technicians with longer formal AI training. The effect of bulls on CR, although ranging from 25.0 to 44.7%, was not significant (Table VIII).

TABLE V. EFFECT OF FACTORS RELATED TO COWS ON CONCEPTION RATE

Factors related to the cow	No. of services	No. of conceptions	Conception rate (%)	Chi-square test
Service number				
1	88	31	35.2	5.324^{ns} (df = 2)
2	12	3	25	
3	16	6	37.5	
4[a]	5	3	60	
Parity number				
1	30	7	23.3	3.81^{ns} (df = 3)
2	51	18	35.3	
3	16	7	43.8	
>4	23	11	47.8	
Heat signs				
Bellowing[a]	4	1	25	1.03^{ns} (df = 1)
Mounting others	36	16	44.4	
Mucus[a]	9	1	11.1	
Restless[a]	2	1	50	
Standing	70	24	34.3	
Body condition score at AI				
3[a]	2	0	0	1.39^{ns} (df = 2)
4	26	12	42.9	
5	38	15	39.5	
6	51	16	31.4	
Lactation state				
Milking & suckling	82	24	29.3	6.3^{*} (df = 1)
Milking only	35	19	54.3	
Degree of uterine tone				
Marked	79	31	37.2	0.53^{ns} (df = 1)
Slight	40	12	30.0	
Swelling of vulva		29	37.2	0.27^{ns} (df = 1)
Marked	78	14	32.6	
Slight	43			
Milk production (kg)				
4–6	11	4	36.3	14.06^{**} (df = 3)
7–9	66	17	25.7	
10–15	37	15	40.5	
16–20	7	6	85.7	

[a]Not included in the Chi-square analysis; ns = not significant (P >0.05); *significant (P <0.05); **highly significant (P <0.01)

3.5. Interventions

The reproductive performance of cows after the interventions were carried out on four farms is shown in Table IX. There was an improvement in the reproductive efficiency after interventions when compared with the reproductive parameters obtained from the survey, with increases ranging from 23.1% to 56.3%.

TABLE VI. EFFECT OF FACTORS RELATED TO THE FARM ON CONCEPTION RATE

Factors related to the farm	No. of services	No. of conceptions	Conception rate (%)	Chi-square test
Region				
Batu Pahat	18	5	27.8	4.3[ns] (df = 3)
Jasin	47	21	44.7	
Kluang[a]	3	2	66.7	
Labis	18	4	22.2	
Merlimau	30	11	36.7	
Feeding system				
Grazing + Conc	47	22	46.8	2.84[ns] (df = 1)
Above + Roughage	67	21	31.3	
Time of AI				
AM	76	30	39.5	1.38[ns] (df = 1)
PM	45	13	28.9	
Interval from heat signs to AI				
6 hours	67	20	29.9	2.12[ns] (df = 1)
12 hours	26	12	46.2	
Time spent on dairying (%)				
26–50	11	3	27.3	0.79[ns]df = 2)
51–75	23	8	34.8	
76–100	80	32	40	

[a]Not included in the Chi-square analysis; ns = not significant (P >0.05)

TABLE VII. EFFECT OF FACTORS RELATED TO THE AI TECHNICIAN ON CONCEPTION RATE

Factors related to AI technician	No. of services	No. of conceptions	Conception rate (%)	Chi-square test
AI technician				
1	23	9	39.1	2.8[ns] (df = 5)
2	47	21	44.7	
3	12	4	33.3	
4	15	4	26.7	
5[a]	6	2	33.3	
6[a]	5	0	0	
7[a]	4	0	0	
8[a]	5	1	20	
9[a]	3	2	66.7	
Site of semen deposition				
Cervix	47	8	17	12.54** (df = 1)
Uterus	71	34	47.9	
Method of thawing semen				
Cold water	49	16	32.7	0.6[ns] (df = 1)
Warm water	68	27	39.7	
Length of formal training				
1 month	31	7	22.6	3.83[ns] (df = 2)
2 months	39	15	18.5	
3 months	47	21	44.7	

[a]Not included in the Chi-square analysis; ns = not significant (P >0.05); **significant (P <0.01)

TABLE VIII. EFFECT OF DIFFERENT BULLS ON CONCEPTION RATE

Bull number	No. of services	No. of conceptions	Conception rate (%)	Chi-square test
248	22	7	31.8	2.08[ns] (df = 4)
276	9	3	33.3	
461	15	6	40	
464	47	21	44.7	
908	16	4	25	

ns = not significant (P >0.05)

TABLE IX. REPRODUCTIVE PERFORMANCE OF DAIRY COWS AFTER IMPLEMENTATION OF INTERVENTIONS

	Farm				Overall mean	Improvement over data from survey (%)
	A	B	C	D		
Number of animals	3	3	4	5		
Calving to first AI (days)	62.7	62	64.5	57.2	61.2	32.5
Calving to conception (days)	90	83	90.5	85.5	87.3	23.1
Services/conception	2	1.6	1.7	1.8	1.8	36.7
Conception rate (%)					55.5	56.3

4. DISCUSSION

4.1. Reproductive status based on milk progesterone concentrations

The finding that a high proportion (93%) of cows had low P4 concentration in milk at the time of insemination indicates that they were likely to have been in oestrus. This suggests that the majority of farmers possessed knowledge or experience in oestrus detection and were using their skills to correctly detect cows in heat. However, there were still instances where cows were wrongly detected in oestrus as evidenced by high progesterone values or cows that were of doubtful status but still bred. The occurrence of such cases (7%), however, was substantially lower when compared to that reported in a previous study [3], where 20% of AIs were done during the luteal phase. The high proportion of cows with ovulatory oestrus suggests that many of the cows under smallholder dairy farming at this location had normal ovarian function, with low occurrence of ovarian disorders such as anoestrus, anovulation, short luteal phases and luteal cysts. The occurrence of these ovarian disorders reported in previous studies was around 12% [4, 5].

The overall CR obtained in this study was lower (35.5%) than that reported in Myanmar [7] and Vietnam [7], but higher than that reported in Cuba [8]. The low CR obtained in the present study can be associated with high occurrence (37.2%) of reproductive wastage, which was similar to that reported in a previous study [9]. It is generally accepted that reproductive wastage caused by embryonic mortality after natural mating or AI accounts for the majority of reproductive failures in cattle, with mortality rates up to 40% of all fertilized eggs [4].

The 100% accuracy of early non-pregnancy diagnosis through milk progesterone RIA was similar to that reported in other studies [7, 9, 10], but the 75% accuracy of early pregnancy diagnosis was lower than the 80–95% range reported in those studies.

4.2. Factors affecting conception rate

The mean intervals from calving to first service (91 days) and to conception (114 days) obtained in this study were considered satisfactory and can produce a calving interval of less than 400 days. This interval to conception is comparable to those obtained in Vietnam, China and Myanmar (110–118 days) and is lower than that obtained in other countries such as Bangladesh, Pakistan, Sri Lanka and Indonesia, which reported intervals of 147–255 days [8]. Variations in the calving to conception interval can be due to many factors especially those related to the cow (such as resumption of postpartum ovarian activity) and management.

A first service CR above 60% is normally considered as an indication of good reproductive efficiency of a dairy herd. On this basis, the result obtained in the present study (35.5%) reflects an unsatisfactory reproductive efficiency. It is lower than the average first service CR of 40.9% obtained in fourteen countries in Asia and Latin America reported in a previous study [8].

In the present study, the factors that had significant effects on CR were milking management (whether it included suckling or not) and level of milk production. However, other factors related to the cow could also have effects on CR. In a previous study based on a large number of observations, CR was found to be significantly higher in cows with marked uterine tone, marked vulval swelling and good body condition [10]. However, other studies have obtained conflicting results on the effects of degree of vulval swelling on CR [6, 7]. In the present study CR tended to increase with increasing parity number, which was similar to the findings in some studies [6, 7], but contradictory to that in one study [11] where CR was significantly higher in cows of first than of fourth parity.

Decreased CR in cows subjected to milking and suckling observed in the present study was also reported in a previous study [7]. The stimulus of suckling is known to have a negative influence on postpartum reproductive functions [4]. It can also drain out more nutrients, causing mobilization of body reserves and negative energy balance. This will result in reduction of body condition if the cow is not receiving adequate nutrition. The BCS is an indicator of the nutritional status of the cow and exerts a marked influence on fertility [12, 13]. It was reported that CR was lower (36% compared with 64%) when cows were inseminated at BCS of 1.0–2.0 than at 3.5–5.0 [10]. In the present study, a BCS of 4 at AI appeared to be optimum for improved fertility, while BCS of 6 indicated obesity or fat cow syndrome, which should be avoided because it can reduce fertility. Besides exerting adverse effects on fertility, obesity could lead to higher occurrence of metabolic, infectious and digestive disorders [12].

The effect of milk production on conception is conflicting. The positive effect of high milk yield on CR in the present study was similar to findings in some previous studies [10, 14], but was in contrast with others [7, 9]. Greater attention given by the farmer to the feeding and management of higher yielding cows could be the reason for the favourable effect on CR [10]. Conversely, it has been reported that CR was inversely proportional to milk yield and that reduced fertility was associated with negative energy balance resulting from the failure of cows to keep pace with the energy demand for high milk production especially during early lactation stage [12]. Decreased CR in cows with high milk production was also observed in other studies [7, 9].

Of the factors related to the farm, the feeding system appeared to be the most important, possibly because milk production and reproductive efficiency are dependent on nutritional status. Cows on adequate nutrition usually maintain satisfactory body condition and positive energy balance which has a favourable influence on fertility [5, 13, 15]. A feeding system that consists of grazing, concentrate and roughage as reported in the present study presumably provided adequate nutrition, especially in energy, which contributed to the improved CR relative to the regime which consisted of only grazing and concentrate supplementation.

Inseminating cows 6 h after detection of standing heat resulted in reduced CR when compared with insemination done after 12 h. Probably 6 h was too short in relation to the time of ovulation. It has been suggested that cows should be bred 12–18 h after detection of heat [16] so that semen can be deposited in the uterus close to the time of ovulation. The relatively higher CR in AI carried out before noon indicates a more conducive environment for conception to take place due to the cooler ambient temperature, compared with AI in the afternoon when the temperature is normally higher. The environmental temperature on the day following AI could also affect conception, as the CR has been observed to be significantly reduced when it exceeded 23°C [11]. High environmental temperature could result in elevated body temperature, resulting in early embryonic death during cleavage stages. Other studies have also reported higher CR when AI was carried out before noon than in the afternoon [7]. However, a study in Myanmar did not find a difference in CR between AI carried before noon or after noon, and when it was done 6 or 12 h after detection of heat [6].

In the present study, the higher CR obtained in the Jasin region compared with that in other regions was apparently not due to the geographical effect of region *per se* but might be associated with the effect of other confounded factors such as feeding system, AI technicians and proportion of time spent by the farmers on dairying activities. Being the first and more established dairy colony in the country, Jasin has many dairy farmers who are more knowledgeable on dairying and many farmers who spend more than 75% of their time on dairying activities compared to other regions. It is likely that they give more attention to the feeding, heat detection and breeding of their animals, which could have contributed to the higher CR. Another reason for the higher CR in the Jasin region is due to the higher CR obtained by the AI technician (# 2) who served the cows in this region.

Even though there were no significant differences in the CR achieved by each AI technician, the figures reflect their degree of skill and experience in the AI technique. As shown in the present study, increased CR was obtained when the AI was carried out by technicians with longer duration of AI training. This implied that longer training improved the skill of the AI technicians. The less skilful technicians might have performed improper inseminations such as depositing semen in the cervix, which would contribute to a decrease in CR. Previous studies also reported a significant increase in CR when semen was deposited in the uterus rather than in the cervix [6, 9, 11]. It is likely that more live spermatozoa can reach the fertilizing site in a shorter time when semen is deposited in the uterus, hence increasing the chances of conception. It has been a recommended practice and a rule of thumb that frozen semen should be thawed in warm water before insemination, and this practice has improved the CR compared to thawing semen in cold water.

The effect of bulls on fertility has long been known to be important and CR can vary from 14.3–80% [11] among individual bulls. Although no significant difference in CR among bulls was observed in the present study, the variation in CR suggests an influence of bulls on fertility. Other factors such as breed of bull, type and attributes of semen have been shown to have significant effects on CR [10]. However, these factors were not determined in the present study.

It is concluded from the results of the survey that there was a high occurrence of ovulatory oestrus in cows presented for AI under smallholder dairy production systems, but the CR obtained from AI was low. CR was, to different degrees, influenced by factors related to the cows, farms, AI technicians and bulls.

The interventions undertaken on four farms resulted in improvement of reproductive performance. Keeping of records on important reproductive events made the farmers more aware of the importance of reproductive management, such as when to start looking for oestrus and when to breed the animal. Ensuring that heat detection is done twice daily beginning thirty days after calving resulted in reduction of undetected oestrus, leading to shorter intervals from calving to first AI and conception. Supplementation of good quality feed from one month prior to and for two months after calving apparently improved the nutritional status of the cows, resulting in increased CR and thus reducing the number of services per conception. This study has been a good demonstration to the farmers that improvement in reproductive performance can be achieved through such practices.

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EVALUATION OF REPRODUCTIVE PERFORMANCE OF CATTLE BRED BY ARTIFICIAL INSEMINATION IN MYANMAR THROUGH THE USE OF PROGESTERONE RADIOIMMUNOASSAY

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Abstract

EVALUATION OF REPRODUCTIVE PERFORMANCE OF CATTLE BRED BY ARTIFICIAL INSEMINATION IN MYANMAR THROUGH THE USE OF PROGESTERONE RADIOIMMUNOASSAY

The productivity and reproductive efficiency of cattle were determined in small-scale farms of upper and lower Myanmar, before and after the introduction of interventions, which included training workshops for farmers, refresher courses for artificial insemination (AI) technicians and quality control of frozen semen. The conception rate (CR) to first service increased from 50% before interventions to 75% after interventions, while the mean calving to first service interval (CFI) and the mean calving to conception interval (CCI) remained unchanged at around 110 days and 125 days, respectively. Supplementary feeding of cows and heifers with urea molasses multi-nutrient blocks (UMMB) during late pregnancy and the post-partum period resulted in a significant reduction in the interval from calving to onset of ovarian activity and a significant increase in the monthly milk yield. It also reduced the CCI and increased growth rate of calves but the differences were not significant. The economic effects of feeding UMMB were studied on six pilot farms by comparing the feed costs with the income from milk in groups of supplemented and control cows. The cost: benefit ratios ranged from 1:1.4 to 1:7.2 in the different farms. A comparison of reproduction in small-scale farms at three locations and medium scale farms at one location showed that CFI ranged from 113.5 ± 31.4 to 133.6 ± 35.2 days in the former and averaged 192.4 ± 95.7 days in the latter. The CR to first service ranged from 58.2% to 81.1% in small-scale farms and was 52.9% in medium scale farms. At all locations, the CFI and CCI were longer and the CR was lower following the first calving than those following the second and later calvings.

1. INTRODUCTION

The national development programme of Myanmar has recognized the need to improve the livestock sector as a means of improving the livelihood of small farmers and concurrently achieving self-sufficiency in livestock products, particularly milk and its products. Dairy farmers in Myanmar are predominantly small-scale, with 1–3 breedable cows, which are kept tethered in a shed or on limited grazing lands. Milking is done by hand, once or twice per day, with the calf as a stimulus to initiate milk let-down.

There is a need in the Asian region for transferring appropriate improved methods for feeding, breeding and management of dairy cattle to the small-scale farmers. Poor oestrus detection is often a major cause of poor reproductive performance [1]. Prolonged post-partum anoestrus is another major cause of economic losses in cattle production in tropical countries [2]. Duration of this period is influenced by stressors such as high temperature, suckling and over-crowding [3]. Several researchers have attempted to induce or synchronize oestrus in post-partum cows by using hormonal therapy [4]. However, it is recognized that the most important factors to consider in attempts to improve fertility are accurate detection of oestrus, correct timing of insemination and providing appropriate management and feeding to permit fertilization, early embryonic development and maintenance of pregnancy [5].

Under improved farm conditions, fertilization rates as high as 75% can be achieved, but by day 40 after AI only 53% of cows are likely to be pregnant [6]. Nutritional status and management are suggested as causative factors. Previous studies have shown that supplementary feeding with urea molasses multi-nutrient blocks (UMMB) increased body weight gain, survival of young, milk production and reproductive performance in cattle, buffalo and sheep [7, 8]. A further study [9] found that supplementation with UMMB reduced the interval from calving to first oestrus and increased fertility. Another aspect that needs to be considered for increasing reproductive performance is the management of breeding bulls and the processing and handling of semen in artificial insemination (AI).

The overall objective of this study was to improve productivity and reproductive efficiency of cattle that are bred by AI, using progesterone measurement by radioimmunoassay (RIA) and recording of field and laboratory data using the Artificial Insemination Database Application for Asia (AIDA Asia). The specific objectives were to: (a) evaluate the reproductive efficiency of cattle in different regions of Myanmar and determine the factors affecting reproductive efficiency on small and medium scale dairy farms by using RIA; and (b) test the effects of feed supplementation with urea molasses multi-nutrient blocks (UMMB) in cows and heifers on growth, milk production and resumption of post-partum ovarian activity.

2. MATERIALS AND METHODS

2.1. Study 1: Reproductive performance of cattle in small-scale farms of upper and lower Myanmar

2.1.1. Animals, management and records

This study was conducted from 1999 to 2002 on small-scale farms in the upper and lower regions of Myanmar. Feeding was based on the ‘cut and carry’ system and hand milking was done twice a day in all farms. Heat detection was based on visual signs observed in the morning and evening, and breeding was done by AI, with no back-up bulls.

During the first two years (1999–2000) the reproductive status of cows was monitored without any interventions. Subsequently, improvements were made by training farmers on management, feeding and heat detection. The AI technicians participating in the study were provided refresher training and the quality of frozen semen supplied from the AI centres was checked to ensure that all batches had post-thaw motility greater than 30%. The study was continued for a further period of two years (2001–2002).

Cows receiving a first AI after calving were included in the study. Data relating to the farms, the inseminated cows, the inseminator and the semen batch were recorded on forms in accordance with the protocol for AIDA Asia [10], which was supplied by the Joint FAO/IAEA Division, Vienna. The forms were returned to the laboratory and the data was entered in to the AIDA Asia software. Ten AI technicians participated in the project and assisted with the collection of data and samples. The total number of cows recorded was 95 in 1999, 302 in 2000 and 476 in 2001–2002.

2.1.2. Collection of milk samples and radioimmunoassay

Milk samples were collected for progesterone RIA at the time of AI (day 0), and on day 10–12 and day 22–24 after AI. The samples were collected equally from the four teats by stripping into 10 mL glass bottles containing 0.1 g sodium azide as a preservative. The samples were transported to the laboratory and centrifuged at 2500 rpm for 15 minutes. After removal of the cream layer by aspiration, the fat free fraction was stored at –20°C until assayed for progesterone.

Progesterone concentration was determined by RIA using a solid-phase system employing a monoclonal antibody and ^{125}I labelled progesterone tracer (FAO/IAEA 'Self-coating' assay). The radioactivity in the antibody-bound fraction was measured using a single well manual gamma counter. Assays were set up with milk samples in duplicate, together with tubes for total counts, non-specific binding, standards prepared in skim milk (0–40 mol/L) and quality control samples. Progesterone values in milk samples were classified as low when they were <1 nmol/L (indicating absence of luteal activity), high when >3 nmol/L (indicating presence of luteal activity) and intermediate when 1–3 nmol/L (indicating a doubtful result).

All data were entered in the AIDA Asia software and summarised using the tabulations available in the application. Selected datasets were exported to MS Excel and analysed further using the Systat statistical package.

2.2. Study 2: Effect of UMMB supplementation on milk yield and reproduction

The first trial was conducted on a medium scale farm in Shwepazon. Ten Friesian crossbred cows between 1st and 4th parity, which were due to calve during a two-month period, were randomly divided into two groups. All cows were fed 2 kg sesame cake and had free access to chopped straw and grass. Cows in group A (n = 5) were supplemented with UMMB at 0.3 kg/cow/day, starting from one month before calving until 12 months after calving. Cows in group B (n = 5) were kept as controls and received no supplementation. Milk production was recorded daily. Milk samples were collected at the time of AI (day 0) and on day 10–12 and day 22–24 after AI.

The second trial was conducted on small-scale farms in the Mandalay region having 1–3 breedable cows per farm. Eighty-three cows that were expected to calve during a two-month period were randomly divided into two groups. Cows in group A (n = 42) were supplemented with UMMB at 0.6 kg/day/cow starting from 14 days before expected calving until 90 days post-partum. Cows in group B (n = 41) were kept as controls.

2.3. Study 3: Effect of UMMB supplementation on post-partum ovarian activity

Eighteen Friesian crossbred cows between 1st and 5th parity, which were due to calve within four weeks, were randomly divided into three groups. All cows were fed concentrate mixture at 1% of body weight, a limited amount of cut grass and chopped rice straw *ad libitum*. Group A (n = 6) were supplemented with UMMB containing 8% urea, group B (n = 6) were supplemented with UMMB containing 12% urea and group C (n = 6) were kept as controls without supplementation. UMMB were fed at 0.5 kg/cow/day from two weeks before calving until 10 weeks after calving.

Milk samples were collected weekly for progesterone assay from days 14–105 post-partum to assess ovarian activity. Assays were done as described previously under section 2.1.2. and post-partum ovarian activity was considered to have started when progesterone concentration in a sample exceeded 1.6 nmol/L [11, 12]

2.4. Study 4: Reproductive performance of cattle in pilot farms and economics of UMMB supplementation

2.4.1. Pilot farms, animals and management

Six pilot farms were established, three in Mandalay (Farms I–III) and three in Yangon (Farms IV–VI). Regionally available agro-industrial by-products were used in the ration whenever available and included cottonseed cake, sesame cake and pea hay.

The numbers of animals studied on each farm and the feed given to each animal were as follows:

- Farm I: 21 cows, randomly divided into two groups (10 + 11). Feed: 3.5 kg sesame cake, 1 kg pea bran, 2 kg pea husk, 1 kg broken rice and 8 kg grass.

- Farm II: 24 cows, randomly divided into two group (11 + 13). Feed: 2 kg sesame cake, 2 kg cottonseed cake, 1 kg pea bran, 1 kg rice bran and straw *ad libitum*.

- Farm III: 22 cows,randomly devided into two group (10 + 12). Feed: 2.5 kg sesame cake, 10 kg groundnut hay 0.5 kg broken rice and straw *ad libitum*.

- Farm IV: 14 cows, randomly divided into two group (7 + 7). Feed: 3.3 kg sesame cake, 16 kg coconut cake, 1.6 kg rice bran, 2 kg wheat bran, 2.3 kg pea husk and grass *ad libitum*.

- Farm V: 12 cows, randomly divided into two groups (6 + 6). Feed: 3.3 kg sesame cake, 1.6 kg rice bran, 3.3 kg pea husk, and grass *ad libitum*.

- Farm VI: 16 cows, randomly divided into two groups (8 + 8). Feed: 3.3 kg sesame cake, 1.6 kg rice bran, 1.6 kg pea bran and grass *ad libitum*.

2.4.2. Interventions

In addition to the above, all cows in the ‘treated’ group were supplemented with UMMB at the rate of 0.5 kg/head/day from 10 days before calving and throughout the post-partum period, while those in the ‘control’ group were kept without the supplement.

On all the farms, the housing was improved to provide good ventilation, drainage system and a hygienic milking parlour. Every batch of semen was checked at the Yangon and Mandalay AI centres before delivery to the field for AI. Workshops and training courses were held for technicians on the importance of proper AI technique, handling of the AI equipment and frozen semen, early pregnancy diagnosis by using milk progesterone determination and identifying problems in infertile cows. Training was given to farmers on heat detection, farm management and feeding practices.

Data for production, reproduction and management were recorded in the AIDA Asia program between 2000 and 2002. The costs and benefits of UMMB supplementation were calculated for the period.

2.5. Study 5: Reproductive performance of cattle in small and medium scale dairy farms of Mandalay

This study was conducted during 2003 in four locations. Locations 1, 2 and 3 had small-scale farms, while location 4 had medium scale farms. All animals were in tie-stalls with zero grazing (cut and carry system). The feeding regime was based mainly on a daily ration of sesame cake (1–2.5 kg), pea bran (1–1.5 kg), pea husk (1 kg), pea hay (1 kg) and rice straw (*ad libitum*). Hand milking was done twice a day (morning and afternoon) and the cows were suckled after each milking by their calves. A total of 251 cows were included in this study.

Milk samples were collected and progesterone was determined by RIA as previously described in section 2.1.2. All the animals were examined by rectal palpation every month to monitor the ovarian changes. All information concerning the farms, cows, AI technicians, semen batches and progesterone values were recorded using the AIDA Asia program.

3. RESULTS AND DISCUSSION

3.1. Study 1: Reproductive performance of cattle in small-scale farms of upper and lower Myanmar

The average calving to first service interval (CFI), calving to conception interval (CCI) and the conception rate (CR) to first service during three different periods of the study are given in Table I.

TABLE I. CALVING TO FIRST SERVICE INTERVAL (CFI), CALVING TO CONCEPTION INTERVAL (CCI) AND THE CONCEPTION RATE (CR) TO FIRST SERVICE IN SMALL-SCALE FARMS

Year	CFI (days)	CCI (days)	CR (%)
1999 (n = 95)	108.9 ± 33.4	121.9 ± 41.4	50.5
2000 (n = 302)	107.0 ± 37.7	126.2 ± 40.0	50.7
2001–2002 (n = 476)	116.5 ± 28.1	125.3 ± 32.5	75.0

The results showed that the CR was significantly higher ($p < 0.0001$) during the third period (2001–2002), which was after the interventions, than during the previous periods. This CR was also higher than that reported from a previous study done in Myanmar [9]. One of the problems identified in that study was the poor quality of frozen semen supplied to the field for AI. In the present study, all batches of semen were checked randomly and only those having greater than 30% post-thaw motility were delivered to the AI technicians. This may have been one of the reasons for the higher fertility observed in the present study, while other factors could be the improved knowledge and skills of AI technicians and farmers due to the training activities that were conducted.

3.2. Study 2: Effect of UMMB supplementation on milk yield and reproduction

All the animals used in the study calved normally. The average CCI in the UMMB supplemented and control cows at the two locations of the study are given in Table II.

TABLE II. CALVING TO CONCEPTION INTERVAL (DAYS) IN UMMB SUPPLEMENTED AND CONTROL COWS

Location	UMMB supplemented	Control
Shwepazon	177 ± 14.84 (n = 5)	199.6 ± 15.74 (n = 5)
Mandalay	112.09 ± 35.7 (n = 40)	133.09 ± 47.01 (n = 34)

In Shwepazon, where the farm was medium scale, the average milk production of the supplemented and control groups was 8.86 ± 2.4 L/day/cow and 7.77 ± 0.94 L/day/cow, respectively. The monthly average milk production of the two groups is shown in Figure 1. Based on a paired (by month) t-test, milk production of the treated group was significantly ($p < 0.0001$) greater than that of the control group. The CCI of the supplemented group was shorter than that of the control group (Table II) and the average body weight gain of calves in the supplemented and control groups was 0.27 kg/day and 0.21 kg/day, respectively. However, these differences were not significant ($p > 0.05$), due in part to the small sample sizes.

In Mandalay, where the farms were small-scale, two out of 42 cows in the supplemented group were culled before the end of experiment because of pyometra and 7 out of 41 cows in the control group were culled because of metritis, death and transfer to other farms. The difference in the mean CCI between the supplemented and control groups (Table II) was statistically significant ($p < 0.02$).

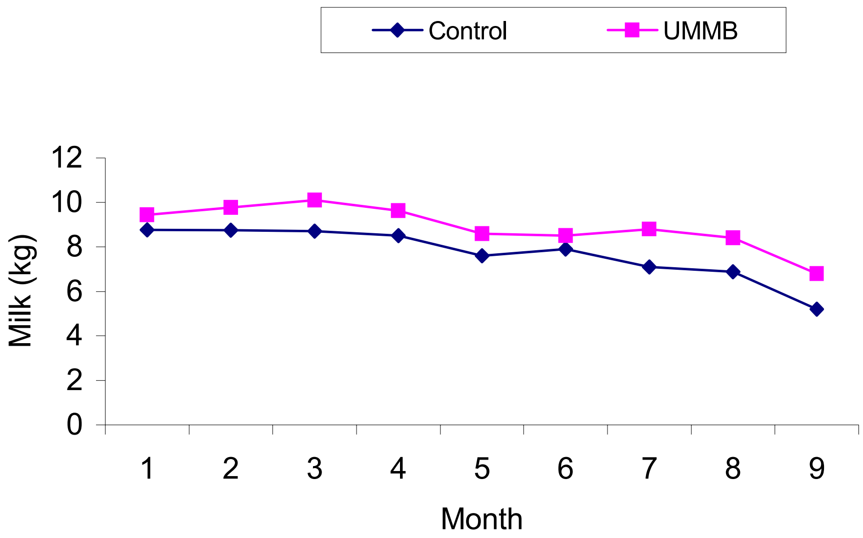

FIG. 1. Monthly average milk production of UMMB supplemented and control cows in the medium scale farm.

3.3. Study 3: Effect of UMMB supplementation on post-partum ovarian activity

The range and mean for the interval from calving to first post-partum ovarian activity in the three groups of cows are given in Table III.

TABLE III. INTERVAL (DAYS) FROM CALVING TO FIRST POST-PARTUM OVARIAN ACTIVITY IN UMMB SUPPLEMENTED AND CONTROL COWS

Group	Range	Mean
A: UMMB, 8% urea (n = 6)	49–91	$78.12 \pm 17.93^{a,b}$
B: UMMB, 12% urea (n = 6)	35–91	60.66 ± 20.60^{a}
C: Control (n = 6)	63–105	92.40 ± 16.17^{b}

^{a,b}Means with different subscripts were significantly ($p < 0.05$) different

Providing increased quantities of urea by supplementation seemed to have a beneficial effect on decreasing the interval from calving to post-partum ovarian activity, although the difference was only significant ($p < 0.05$) between the two most extreme groups (control and 12% urea). These results are similar to previous reports on the effects of UMMB supplementation [13] and indicate the importance of adequate energy and protein in the diet of cows during the post-partum period [14].

3.4. Study 4: Reproductive performance of cattle in pilot farms and economics of UMMB supplementation

The mean CCI, milk production and economic aspects of the feeding regimes for the treatment and control groups in each pilot farm are summarised in Table IV. Based on a paired t-test (paired by farm), the supplementation significantly ($p < 0.02$) decreased the mean CCI. In addition, the average increase in milk production per day associated with supplementation approached significance ($p = 0.06$).

TABLE IV. CALVING TO CONCEPTION INTERVAL (CCI), MILK PRODUCTION, FEED COST AND COST: BENEFIT RATIO IN UMMB SUPPLEMENTED AND CONTROL COWS

Farm	Group	Mean CCI (days)	Mean milk production (L/day)	Feed cost (Kyat/day)	Cost: Benefit
I	UMMB	108 (n = 10)	16.6	600	1:1.5
	Control	127 (n = 11)	16	565	
II	UMMB	115 (n = 11)	10.2	565	1:2.6
	Control	122.8 (n=13)	9.4	385	
III	UMMB	124 (n = 10)	12.33	395	1:1.4
	Control	148 (n = 12)	11.8	360	
IV	UMMB	176.85 (n = 7)	11.43	843	1:7.2
	Control	178.28 (n = 7)	10.57	818	
V	UMMB	132.5 (n = 6)	10.6	675	1:2.5
	Control	143 (n = 6)	10.3	650	
VI	UMMB	143.5 (n = 8)	11.12	645	1:2.1
	Control	155.75 (n = 8)	10.87	620	

Farms I–III, located in Mandalay, were all small-scale farms. Most of the cows were high milk producers and supplementary feeding gave a good production response resulting in a greater profit margin, although milk prices were low. Supplementation with UMMB also enabled the quantity of concentrates fed to be reduced without a loss in milk production, thus saving on feed costs.

Farms IV–VI, located in Yangon, were larger than those in Mandalay and the milk prices were higher. The highest economic benefit of UMMB feeding was observed in farm IV, which was one of the largest private farms in the area.

3.5. Study 5: Reproductive performance of cattle in small and medium scale dairy farms of Mandalay

A total of 418 services were recorded at the four locations during the year of study. Overall, the number of services was lower during the winter season than during the rainy and summer seasons. The mean CFI (days) at locations 1, 2, 3 and 4 were 114.6 ± 30.4 (n = 55), 113.5 ± 31.4 (n = 51), 133.6 ± 35.2 (n = 74) and 192.4 ± 95.7 (n = 55), respectively. The corresponding mean CCI (days) were 119.3 ± 33.4 (n = 41), 118.9 ± 35.1 (n = 36), 141.3 ± 29.7 (n = 54) and 180.6 ± 91.8 (n = 20), respectively.

The CFI and CCI were shorter in small-scale farms (locations 1–3) than in medium scale farms (location 4), and longer following the first calving than those following the second and later calvings at all locations, but the differences were not statistically significant. The numbers of services per conception and the first service CR at the four locations are shown in Table V. Overall, the first service CR was 56% following the first calving and 60% following the second and later calvings.

TABLE V. SERVICES PER CONCEPTION AND CONCEPTION RATE AT FOUR LOCATIONS

Location and type of farm	Services per conception			Conception rate (%)
	After first calving	After second and later calvings	Overall	
1: Small-scale	1.64 (41/25)	1.57 (47/30)	1.6 (88/55)	81.1
2: Small-scale	1.39 (25/18)	1.24 (41/33)	1.29 (66/51)	70.6
3: Small-scale	1.3 (39/30)	1.27 (56/44	1.28 (95/74)	58.2
4: Medium scale	1.9 (19/37)	1.6 (16/10)	1.8 (53/29)	52.9

The effect of parity on overall reproductive efficiency is probably due to several factors that differ between the first calving heifers and older cows [15]. It is known that the first lactation has a negative effect on fertility, particularly in tropical climates [16].

Measurement of progesterone in samples collected from 43 animals in small-scale farms showed that all animals had low progesterone on the day of AI, indicating that services were likely to have been done at the appropriate time. Of these, 24 animals (55.8%) had high progesterone concentrations on days 10–12 and 22–24 after AI, indicating that they had probably conceived. Of the latter, 3 animals (12.5%) were later found to be non-pregnant by rectal palpation, indicating that they may have undergone late embryo death.

Overall, 54.2% of the milk samples had intermediate concentrations of progesterone (1–3 nmol/L), which could be due to individual variations in the oestrus cycle, handling of the samples or variability of the assay results. This emphasises the need for rigorous control at each stage of the sampling and assay process for accurate interpretation of the results from progesterone measurement [17].

4. CONCLUSION

This study has shown that reproductive performance is lower in heifers than in cows and that management and nutrition had important effects on fertility after AI. Supplementation with UMMB resulted in shorter intervals from calving to onset of ovarian activity, increased milk production in cows and increased body weight gain in calves. The interval from calving to first service was shorter in small-scale farms than in medium and large farms. 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. Ensuring that the frozen semen supplied for use in AI was of good quality improved CR. Measuring the concentration of progesterone in milk was very useful for evaluating the status of ovarian activity.

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IMPROVING REPRODUCTIVE EFFICIENCY IN AN ARTIFICIAL INSEMINATION PROGRAMME THROUGH EARLY NON-PREGNANCY DIAGNOSIS, MANAGEMENT AND TRAINING

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Abstract

IMPROVING REPRODUCTIVE EFFICIENCY IN AN ARTIFICIAL INSEMINATION PROGRAMME THROUGH EARLY NON-PREGNANCY DIAGNOSIS, MANAGEMENT AND TRAINING

A field survey was conducted on a stratified random sample of the owners of 300 buffaloes and 200 cattle. The main buffalo farming systems were rural subsistence, rural market-oriented, peri-urban and commercial farming, while the main cattle farming systems were rural rain-fed (Barani), rural irrigated, peri-urban and progressive farming. In buffaloes the average age of maturity (first use for breeding) was 34.8 ± 5.7 months and the calving interval (CI) was 17.5 ± 4.7 months. In cattle the average age at maturity was 32.4 ± 6.4 in purebreds, 20.1 ± 7.2 in crossbreds and 35.5 ± 10.4 in indigenous animals, while the CI was 15.9 ± 5.1, 13.1 ± 2.8 and 12.3 ± 6.8 months for the three breed types, respectively. Milk samples were collected for from 137 cattle and buffaloes that were subjected to artificial insemination (AI) on the day of AI (day 0), and on days 10–12 and 21–23 after AI. The average concentration of progesterone was 0.15 ± 0.02 ng/mL in milk collected on day 0 and 3.26 ± 0.29 ng/mL in those collected 10–12 days later. Based on results from the first two samples, 77% of cows had a low-high progesterone combination, indicating that they had been inseminated at an ovulatory oestrus. Based on results from all three samples, 42% of cows had a low-high-high progesterone combination and were subsequently diagnosed as pregnant by rectal palpation. A low-high-low progesterone combination was observed in 19% of cows and all were found non-pregnant on rectal palpation. It was concluded that milk progesterone assay is effective of the early diagnosis of non-pregnancy in cattle and buffaloes.

1. INTRODUCTION

Livestock production is an important sub-sector of Pakistan's agriculture and the country has some of the finest breeds of buffaloes (Nili-Ravi and Kundi) and cattle (Sahiwal, Red Sindhi and Cholistani), along with several draught breeds. The economic viability of farming systems that sustain livelihoods through livestock farming demands optimum reproductive performance. Maintaining high fertility in the herd by ensuring that reproductive events are kept on schedule is of great importance. Long calving interval (CI) means less milk to market, increased cost of production, slower genetic improvement and fewer herd replacements. The successful implementation of emerging technologies can only be built upon a solid foundation of basic reproductive management.

The principal objectives of artificial insemination (AI) in dairy farming are genetic improvement, control of venereal diseases and optimum reproductive performance. At present about 5.8% of buffalo and 22.3% of cattle in Pakistan are bred by AI, while the rest of the breedable animals are bred by natural service. The overall conception rate (CR) from AI ranges from 38–62% [1]. The CR in indigenous cattle and buffaloes is very low due to poor awareness of farmers, low quality feed, long distances between the farms, inappropriate detection of heat, insemination at the wrong time and unskilled technicians [2].

Early diagnosis of cyclicity and pregnancy in cattle and buffaloes, coupled with training of farmers and A.I technicians, can improve the reproductive efficiency. Measurement of progesterone levels in milk has been used for the detection of luteal activity, early non-pregnancy diagnosis and assessing oestrus detection [3]. Buffaloes monitored for oestrus by determining milk progesterone levels achieved a CR of 85%, whereas those inseminated on the basis of clinical signs of heat alone had a CR of 52%. [4].

The specific objectives of this project were to: (a) assess the accuracy of milk progesterone radioimmunoassay (RIA) for early diagnosis of non-pregnancy; (b) identify the main bottlenecks for improving productivity under different production systems; (c) improve the fertility through treatment of genital diseases; and (d) conduct refresher training in AI, productive and reproductive management of dairy animals for in-service veterinarians, AI technicians (AITs) and farmers.

2. MATERIALS AND METHODS

2.1. Non-pregnancy diagnosis

This study was conducted at two AI centres, namely Lahore and Burki. Milk samples (5 mL) were collected from 137 animals including 130 cows and 7 buffaloes on the day of AI (day 0) and days 10–12 and 21–23 after AI, into glass tubes containing a sodium azide tablet as a preservative. The milk samples were centrifuged at 2000x g for 20 minutes at 4ºC, the fat was separated and the skim milk samples were stored at –20ºC until analysed. The progesterone concentration was determined by the 'Self-coating RIA' method using the reagents and protocol provided by the Joint FAO/IAEA Division of Nuclear Techniques, Vienna (Bench Protocol Version Sc-RIA 3.1., 1999).

Progesterone concentrations below 0.1 ng/mL were categorized as low and considered as indicative of non-luteal phase (i.e., oestrus or anoestrus) [5], those between 1.0–3.0 ng/mL were categorized as intermediate and those above 3.0 ng/mL as high (i.e. luteal phase or pregnancy).

2.2. Survey

A survey to assess the problems of farmers and to identify the constraints in improving productive efficiency was conducted by the University of Agriculture, Faisalabad, through a questionnaire, personal interviews and the Participatory Rural Appraisal (PRA) technique. Out of 34 districts of the Punjab, 9 were selected using stratified random sampling based on the percentage of animals reported for each species. The same percentages were used for selecting stratified samples for various production systems in each species. The owners of 300 buffaloes and 200 cattle were selected to carry out the survey.

2.3. Improving fertility in dairy animals

2.3.1. Effect of UMMB supplementation on resumption of post-partum cyclicity

Sixteen buffaloes were selected for this study, 12 from the Ayub Agriculture Research Institute (AARI), Faisalabad, and 4 from a private farm situated on Jhang Road, Faisalabad. The animals were divided into two groups of eight, those that had calved within one month (Group I) and those that had calved between three to four months earlier (Group II). All animals were allowed free access to available green fodder and received cottonseed cake mixed with wheat straw at a ratio of 1:3. In each group, 4 animals were provided with UMMB at an average of 400 mg/day, while the other 4 animals were kept as controls and received no supplement. Milk samples were collected weekly and the concentration of progesterone was determined by the 'Pre-coated Tube RIA' method (Diagnostic Products Corporation, USA), using the reagents and protocol provided by the Joint FAO/IAEA Division of Nuclear Techniques, Vienna (Bench Protocol Version 2.0, January 1993). The data was analysed according to Duncan's Multiple Range test.

2.3.2. Reproductive health management

A total of 155 buffaloes and cows that were brought to the outreach centre of the University of Agriculture, Faisalabad, which had been diagnosed as having endometritis on the basis of history, rectal and vaginal examination and clinical observations, were given the following treatments.

Sixty-three buffaloes and cows that had a corpus luteum (CL) on either of the ovaries were each injected with 500 ug of cloprostenol intramuscularly on day 7 of the oestrus cycle (day of oestrus was designated as day 0). The animals were examined clinically at the first oestrus following treatment and were inseminated with fresh diluted semen.

Buffaloes and cows that were diagnosed to be suffering from first-degree endometritis were inseminated at the time of oestrus. Twenty-four hours after insemination, each animal was given an intrauterine infusion of a combination of procaine penicillin (4 million I.U.) and streptomycin (5 g) or gentamycin sulphate (600 mg). For animals suffering from second-degree endometritis, samples of uterine secretions were obtained and subjected to culture and sensitivity testing, and the appropriate antibiotics were administered through intrauterine infusions for 5–7 days.

2.3.3. Effect of refresher training of inseminators on conception rate

Five professional degree holders and 10 AITs were subjected to evaluation of their insemination skills using coloured water to perform simulated AI in animals intended for slaughter. Five AITs were unsuccessful and were sent for three days of intensive practical training at the Livestock Services Training Centre, Sheikhupura. The performance of these AITs was recorded one month before and after training.

3. RESULTS

3.1. Non-pregnancy diagnosis

Results from progesterone measurement in all three milk samples (days 0, 10–12 and 21–23) were available for 105 cows. They were all examined by rectal palpation for pregnancy diagnosis 60–90 days after AI. The findings and interpretation are shown in Table I. The overall conception rate was 42%. The 44 animals that conceived were all among the 61 cows that showed a low-high-high progesterone combination. A low-high-low progesterone combination was observed in 20 cows (19.1%) and all were found non-pregnant on rectal examination. Three cows (2.9%) were inseminated while pregnant. Considering the results from samples collected on days 0 and 10–12, 81 cows (77%) had a low-high progesterone combination, indicating that they had been inseminated at an ovulatory oestrus.

TABLE I. RESULTS ON PROGESTERONE CONCENTRATION IN THREE CONSECUTIVE MILK SAMPLES FOLLOWED BY RECTAL PALPATION IN 105 COWS

Progesterone concentration			Rectal palpation	Number of cows (%)	Interpretation
Day 0	Day 10–12	Day 21–23			
Low	High	High	Positive	44 (41.9)	Pregnant
Low	High	High	Negative	17 (16.2)	Late embryonic mortality, luteal cyst, persistent CL
Low	High	Low	Negative	20 (19.1)	Non-fertilization, early embryonic mortality
High	High	High	Positive	3 (2.9)	AI on pregnant animal
*	*	*	Negative	21 (20.0)	Other clinical data is required for proper interpretation

*At least one of the samples showed an intermediate value (1–3 nmol/L)

3.2. Survey

The survey showed that buffalo farming is practiced under four different production systems, namely rural subsistence (RS), rural market-oriented (RMO), peri-urban (PU) and commercial (Com) farming. The cattle production systems were rural rain-fed (termed rural barani, RB), rural irrigated (RI), peri-urban (PU) and progressive (Prog) farming.

3.2.1. Age at maturity and calving interval

The age at maturity (defined as the age when farmers stated that females were first used for breeding) and CI of buffaloes under different production systems are given in Table II. Both the age at maturity and CI were lower in the Com system.

The average age at maturity and CI of cattle under different production systems are given in Table III. The highest age at maturity was observed in non-descript cattle and the lowest in crossbred cattle. The highest CI was observed in purebred cattle and the lowest in non-descript cattle. The causes of late maturity and long CI were investigated through discussions and the perceptions of farmers about the causes in buffaloes and cattle are presented in Table IV.

TABLE II. AVERAGE AGE AT MATURITY AND CALVING INTERVAL IN BUFFALOES UNDER DIFFERENT PRODUCTION SYSTEMS

Production System	No. of animals surveyed	Age at maturity (months)	Calving interval (months)
Rural subsistence	72	34.2 ± 4.4	18.4 ± 5.5
Rural market-oriented	196	35.6 ± 5.9	17.2 ± 5.0
Peri-urban	15	33.7 ± 5.1	16.9 ± 9.1
Commercial	13	27.6 ± 5.0	5.2 ± 2.9
Overall	256	34.8 ± 5.7	17.5 ± 4.7

TABLE III. AVERAGE[a] AGE AT MATURITY AND CALVING INTERVAL IN CATTLE UNDER DIFFERENT PRODUCTION SYSTEMS

Breed type	Parameter	Production system				Overall
		Rural barani[a]	Rural irrigated	Peri-urban	Progressive	
Purebred	Age at maturity	33.4 ± 3.9	31.9 ± 7.3	33.0 ± 4.2	–	32.4 ± 6.4
	Calving interval	15.1 ± 4.1	16.5 ± 5.7	15.7 ± 2.1	–	15.9 ± 5.1
Crossbred	Age at maturity	15.6 ± 2.6	22.7 ± 8.0	19.8	27.0	20.1 ± 7.2
	Calving interval	13.5 ± 2.7	12.9 ± 2.9	13.4 ± 3.1	12.0	13.1 ± 2.8
Non-descript	Age at maturity	37.0 ± 1.4	35.4 ± 11.2	36.0	–	35.5 ± 10.4
	Calving interval	17.2 ± 4.5	11.9 ± 6.7	15.0	–	12.3 ± 6.8

[a]In months.
[b]Rural rain-fed

TABLE IV. PERCENTAGE OF FARMERS REPORTING DIFFERENT CAUSES FOR LATE MATURITY AND LONG CALVING INTERVAL IN BUFFALOES AND CATTLE

Parameter	Reasons	Buffaloes (%)	Cattle[a] (%)
Age at maturity	Breed character	26.4	51.6
	Poor nutrition	65.8	43.8
	Diseases	4.1	3.9
	Management	3.7	0.7
Calving interval	Breed character	35.1	51.6
	Poor nutrition	45.5	43.8
	Diseases	11.9	3.9
	Management	7.5	0.7

*Results were the same for both age at maturity and calving interval in cattle

3.2.2. Reproductive disorders

The prevalence of reproductive disorders in the different production systems for buffaloes is given in Table V and for cattle in Table VI.

TABLE V. PREVALENCE OF REPRODUCTIVE DISORDERS IN BUFFALOES IN DIFFERENT PRODUCTION SYSTEMS

Disorder	n	Production system[a]				Overall (%)
		RS (%)	RMO (%)	PU (%)	Com (%)	
Anoestrus	125	49.3	47.7	33.3	41.7	47.0
Silent heat	89	32.7	32.6	46.7	33.4	33.5
Metritis	24	9.0	9.2	6.7	8.3	9.0
Calving difficulty	18	6.0	6.4	13.3	8.3	6.7
Dystocia	10	3.0	4.1	–	8.3	3.8

[a]RS - rural subsistence; RMO - rural market-oriented; PU - peri-urban; Com - commercial

TABLE VI. PREVALENCE OF REPRODUCTIVE DISORDERS IN CATTLE IN DIFFERENT PRODUCTION SYSTEMS

Disorder	n	Production System[a]				Overall (%)
		RB (%)	RI (%)	PU (%)	Prog (%)	
Anoestrus	51	23.4	41.7	100	–	36.7
Silent heat	6	–	6.0	–	33.3	4.3
Metritis	56	46.8	38.1	–	66.7	40.3
Calving difficulty	18	27.7	6.0	–	–	12.9
Dystocia	8	2.1	8.2	–	–	5.8

[a]RB - rural barani (rain-fed); RI - rural irrigated; PU - peri-urban; Prog - Progressive

3.3. Improving fertility in dairy animals

3.3.1. Effect of UMMB supplementation on resumption of post-partum cyclicity

In Group I, the animals fed UMMB started ovarian activity between 88 and 105 days after calving, as determined from elevated concentrations of milk progesterone (3–5 ng/mL). These animals came into heat, were inseminated and maintained high milk progesterone levels (2.1–6.2 ng/mL), which confirmed that they had conceived. The control animals had basal progesterone concentrations up to 130 days after calving

In Group-II, the animals fed UMMB started cyclicity between 95 and 105 days after calving. They were inseminated and conceived. Animals in the control group started cyclicity between 138 and 172 days after calving. Three of these animals conceived and maintained a high level of progesterone while one animal showed a decline to basal levels 22 days after insemination. There was no significant difference in the elevated progesterone concentrations, which fluctuated in the range of 2–9 ng/mL, between the two groups.

3.3.2. Reproductive health management

Out of 63 animals that were diagnosed with endometritis and having a CL in one of the ovaries, 54 (77.5%) responded to treatment with cloprostenol and showed oestrus signs.

These animals were inseminated with fresh liquid semen and were examined 60–90 days later. Thirty-three animals (52%) were found pregnant. Of the 90 animals that were diagnosed with endometritis and treated with antibiotics, 42 (46.7%) conceived.

3.3.3. *Effect of refresher training of inseminators on conception rate*

The CRs obtained by individual AITs before and after three days of intensive refresher training are shown in Table VII. The overall CR achieved was 23.2% before the training and 37.4% after the training. This difference was highly significant (p <0.002).

TABLE VII. CONCEPTION RATES ACHIEVED BY INDIVIDUAL AI TECHNICIANS BEFORE AND AFTER REFRESHER TRAINING

AI Technician	Before training		After Training	
	No. pregnant/No. of services	Conception rate (%)	No. pregnant/No. of services	Conception rate (%)
# 1	12/50	24.0	19/48	39.6
# 2	14/64	21.9	20/57	35.1
# 3	9/34	26.5	21/46	45.6
# 4	11/45	24.4	15/39	38.5
# 5	3/18	16.7	8/32	25.0
Overall	49/211	23.2	83/222	37.4

4. DISCUSSION

Negligence in oestrus detection is a major contributory factor towards low reproductive efficiency. The results of this study indicated that 25% of cows were inseminated at the incorrect time of the oestrus cycle. These cows were bred either during the luteal phase or too early or too late in relation to oestrus. Rajamahendran *et al.* [6] reported from a study in Canada that 4.8% of cows were inseminated during the luteal phase, while Dzung *et al.* [7] reported from a study in Vietnam that 23% of cows were inseminated either too early or too late in relation to oestrus. The insemination can be considered to have been done at the correct time when progesterone concentration is basal (<0.2 ng/mL) on the day of insemination and elevated (>2 ng/mL) six days later [8].

Determination of progesterone level in milk has been widely used as a way of monitoring CL function. In the present study, 16.2% of the animals had a combination of low-high-high progesterone followed by a finding of non-pregnancy on rectal palpation, indicating late embryonic mortality, persistent CL or luteal cyst. The finding of low-high-low progesterone pattern in 19.1% of the animals indicated either fertilization failure or early embryonic death. Weigelt *et al.* [9] analysed 472 oestrus cycles and recorded 10% embryonic mortality, out of which 1.1% was due to the post conception insemination. Studies have shown that cows with progesterone concentrations of 0.24 ng/mL at the time of insemination showed the best pregnancy rate, while none of those with concentrations above 0.5 ng/mL conceived [10].

The other factors responsible for lowered fertility are managemental and pathological. Among the managemental factors, nutritional management bears a high significance in the location of this study [11]. Poor nutrition was identified as a major cause of late maturity and long CI by the respondents of the survey. The present study on supplementation of feed with

UMMB resulted in earlier commencement of cyclicity after calving. Similar results have been reported in India [12].

Reproductive disorders in female buffaloes and cows cause substantial economic losses. The present survey revealed that the major reproductive problem in buffaloes was anoestrus, followed by silent heat. In cattle, however, the major problem was metritis, followed by anoestrus. Previous studies [13] have indicated an incidence of 58% for true anoestrus in buffaloes, which is higher than the present finding, and an incidence 33% for silent oestrus, which is in close agreement with these results. It is well documented that the incidence of silent ovulation is higher in buffaloes that calve during the low breeding season than in those which calve during the peak breeding season [14].

Metritis has been identified as a major reproductive problem in previous studies, with an incidence of 80% in cattle [15] and 51% in buffaloes based on clinical data [14] and 54% based on slaughter-house material [16]. The uterus is relatively susceptible to infection when blood progesterone concentration is high and is resistant to infection when blood oestrogen concentration is high [17]. The onset of oestrus following cloprostenol injection results in an environment of oestrogen influence and thus enhances phagocytic activity. The results of the present study are in agreement with those reported by Humblot [18] and Zuber [19], where cloprostenol therapy was found to be effective for endometritis. The treatment is convenient, reasonably priced and produces quick results. Treatment of uterine infections with antibiotics proved more beneficial when these were administered on the basis of culture and sensitivity testing to select the appropriate drug. However, under field conditions where this is not feasible, the intra uterine administration of a combination of procaine penicillin and streptomycin proved reasonably effective.

The skills of AITs have an important influence on the success of AI in the field. The AITs are responsible for ensuring that animals for insemination are at the appropriate stage of oestrus, and must handle semen correctly and observe proper hygiene to achieve the optimum results. The three days of intensive refresher training provided to the AITs who had poor skills resulted in a clear improvement of their performance.

In conclusion, the present study has shown the need to increase the awareness of farmers regarding reproductive health management, especially on heat detection and the nutritional requirements for maintenance and production. The skills of AITs need to be evaluated and improved regularly. Their performance should be monitored on the basis of CR rather than number of inseminations done. Milk progesterone assay can be used effectively for early non-pregnancy diagnosis. The problems of anoestrus and silent heat in buffaloes must be addressed.

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IMPROVING THE PERFORMANCE OF ARTIFICIAL INSEMINATION SERVICES THROUGH APPLICATION OF RADIOIMMUNOASSAY AND COMPUTERIZED DATA MANAGEMENT PROGRAMMES

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Abstract

IMPROVING THE PERFORMANCE OF ARTIFICIAL INSEMINATION SERVICES THROUGH APPLICATION OF RADIOIMMUNOASSAY AND COMPUTERIZED DATA MANAGEMENT PROGRAMMES

The performance of the national artificial insemination (AI) service in Sri Lanka was assessed by obtaining and analyzing available data, followed by the conducting of a field survey on a sample of artificially-bred cattle kept by smallholder farmers in the mid-country wet zone region and on large scale farms in the up-country wet zone region. Samples of milk were collected from inseminated cows and the concentration of progesterone was measured by radioimmunoassay (RIA). The AI service reached only 18.8% of the breedable cattle on a national level and accounted for only 6.3% of the estimated annual calvings. The mean intervals from calving to first service and from calving to conception were 183 ± 87.1 days (n = 211) and 194 ± 93.9 days (n = 143), respectively on smallholder farms, and 111.2 ± 74.2 days (n = 133) and 156 ± 92.7 days (n = 170), respectively, in large farms. The average conception rate to first service ranged from 33–50% on smallholder farms and from 31-60% on large farms.

Based on the findings, action was instituted to (a) develop a monitoring system for quality control of frozen semen; (b) develop intervention protocols for management of repeat breeder cows; (c) provide early non-pregnancy diagnosis and infertility management services to large farms; (d) transfer the technologies by conducting continuing professional development (CPD) programmes for field veterinarians and AI technicians; and (e) introduce and institutionalize three computerized data management. Semen donor bulls at the national AI centre were evaluated for breeding soundness and a routine procedure was established for pre- and post-freezing semen evaluation, including the Hypo-osmotic Swelling Test (HOST). The incidence of repeat breeder cows was higher in smallholder farms (15.2%) than in large farms (6.6%). Only one-third of the repeat breeder animals exhibited clear signs of an abnormality. The use of milk progesterone as a routine procedure for early non-pregnancy diagnosis was tested on two large farms and abandoned due to logistical problems. An average of three CPD courses were conducted per year for field veterinarians and technical staff engaged in AI services. Three computer software packages, the Semen Processing Records Management (SPeRM), Artificial Insemination Database Application for Asia (AIDA Asia) and Livestock Information Management Application (LIMA) were introduced to the national AI services to improve the efficiency of recording, analysing and reporting information.

1. INTRODUCTION

The dairy industry plays an important role in the agrarian economy of Sri Lanka. Development of this sector is viewed as a means of reviving the rural economy, achieving national self-reliance and ensuring food security in milk and milk products. Of the many constraints in dairy development, low genetic merit of indigenous cattle and buffaloes has been considered as the primary one. Therefore, genetic upgrading of indigenous stock through crossbreeding with exotic dairy type animals has been pursued vigorously over the past five decades. In this attempt, artificial insemination (AI) has been accepted as the primary breeding method [1]. However, many reviews and consultancy reports on the dairy sector have highlighted the slow progress achieved in genetic upgrading programmes and the poor performance of the national AI service [2–4.].

Therefore, a series of studies was carried out commencing in the mid 1990s to assess the performance of the AI service and identify its constraints, in order to develop and implement remedial measures. Initially, a field survey was done on a sample of artificially-bred cattle maintained by smallholder farmers and large scale state farms. The field observations were complemented with data on measurement of milk progesterone using radioimmunoassay (RIA). The RIA technique provides a very sensitive, precise and robust method of quantifying the minute amounts of progesterone present in blood or milk, which is an indicator to determine the commencement of ovarian cyclicity in post partum cows, monitor the stages of the oestrus cycle and to perform early diagnosis of non-pregnancy [5]. Based on the findings of the initial survey, problems that required further investigations were identified [6, 7] and appropriate activities were undertaken to address the problems. They were further complemented by conducting regular field fertility clinics in conjunction with veterinary undergraduate training in several field locations around the University of Peradeniya.

The overall aim of the project was to improve the productivity of smallholder and large-scale dairy farms through improvement in the performance of AI services provided by the state veterinary service. The specific objectives were to: (a) assess the performance of AI services at national, regional, district and farm levels; (b) develop a monitoring system for quality control of frozen semen; (c) develop intervention protocols for management of 'repeat-breeder' cows; (d) provide early non-pregnancy diagnosis and infertility management services to smallholder and large farms; (e) transfer the technologies by conducting continuing professional development programmes for field veterinarians and AI technicians; and (f) introduce and institutionalize a computerized data management package compatible with existing data recording systems for managing semen, AI and farm data.

2. MATERIALS AND METHODS

2.1. Performance of AI services at national, regional, district and farm levels

2.1.1. Coverage and performance of the national AI service

The performance of the national AI service was assessed by estimating the percentage of breedable cows that are served by AI, calving rate from AI and percentage of calves born from AI. The data for calculating national, regional and district level AI coverage and calving rates were obtained from the official statistics of the Department of Animal Production and Health (DAPH), which is the state institution responsible for veterinary services, livestock breeding and extension.

2.1.2. Success of AI done in smallholdings

The use and effectiveness of AI was assessed through a field-survey and a longitudinal study that were conducted in the mid-country wet zone region (elevation 500–1500 masl). Both activities were carried out in collaboration with the state veterinary officers and AI technicians in five selected 'veterinary ranges', each range being the geographical area of responsibility of a field veterinary officer.

The field survey was conducted using a structured questionnaire that gathered information on herd size and composition, breed types, methods of rearing, feeding, breeding and heat detection, preference of farmers for semen from different breeds, and their perceptions regarding the AI service. A total of 200 smallholdings were surveyed.

The longitudinal study was done in four of the five veterinary ranges that were selected for the field survey. Dairy cows receiving the first AI following a recorded calving were monitored until they were diagnosed as being pregnant. On the day of AI (day 0), detailed information relating to the farm, cow, semen batch and inseminator were recorded using forms developed according to the protocol of the Artificial Insemination Database Application for Asia (AIDA Asia), which was provided by the Joint FAO/IAEA Programme, Vienna [8].

Samples of milk (20 mL) were collected from each cow into tubes containing sodium azide as a preservative on day 0, and on days 10–12 and 21–23 after AI. Dates of subsequent services were recorded for cows presented for repeat AI. In all cases, those not returning to heat within 60–90 days after the last service were examined for pregnancy by rectal palpation. The milk samples were centrifuged to separate the fat fraction and the skim milk was stored at 4°C. Progesterone concentration was determined in skim milk by a solid-phase RIA method using kits supplied by the Joint FAO/IAEA Programme, Vienna [9].

All field and laboratory data were entered into the AIDA Asia database, summarized and analysed using the features of the application.

2.1.3. Success of AI done in large farms

This study was conducted on four large farms (Ambewela, New Zealand, Bopaththalawa and Dayagama) located in the up-country region (elevation 1500–2000 masl), which serve as multiplier farms for providing improved breeding stock to smallholders. A total of 200 cows receiving the first AI after calving were monitored until they became pregnant. Collection of information and milk samples, measurement of progesterone and data analysis were done as described above under 2.1.2.

2.2. Evaluation of semen donor bulls and quality control of deep frozen semen

The breeding soundness of a sample of five bulls that were kept at the Central Artificial Insemination Service (CAIS) of the DAPH located at Kundasale, was evaluated. The procedures for observation, inspection, clinical examination and rectal palpation were conducted according to standard veterinary methods [10]. Semen samples were collected from each bull using an artificial vagina and were examined for volume, colour, density, total sperm concentration, mass and individual motility, live:dead ratio (using Nigrosin-Eosin stain), sperm morphology (using Williams' stain) and percentage of sperm positive for the Hypo-osmotic Swelling Test (HOST). The HOST was performed by adding 0.1 mL of semen to 1.0 mL of hypo-somatic solution and incubating the mixture at 37°C for 40 minutes. The number of HOST positive sperms was counted under a phase contrast microscope and the percentage was calculated.

The semen samples were subjected to the standard procedure for freezing bovine semen at the CAIS, thawed and examined for percentage motility. live:dead ratio, morphology and percentage of HOST positive sperms.

Subsequently, the procedure for pre- and post-freezing evaluation of semen from donor bulls at CAIS was continued on a regular basis as a routine practice.

2.3. Development of intervention protocols for management of repeat-breeder cows

A survey was done on cows that were cycling normally and had received several AI services on smallholder farms and two large farms. Based on the history and rectal palpation, the animals were categorised as follows: (1) received less than three services and found to be pregnant or non-pregnant; (2) received more than three services and found to be pregnant; and (3) received more than three services and found to be non-pregnant. The latter group were termed repeat-breeders and were subjected to further detailed clinical examination, including inspection of the vulva and vagina, and evaluation of the status of the uterus and ovaries by rectal palpation. Samples (n = 100) were collected from the uterus for microbiological culture, transported to the laboratory and cultured on blood agar.

2.4. Early non-pregnancy diagnosis and infertility management services

The application of progesterone measurement in milk samples for early non-pregnancy diagnosis was introduced as a potential routine practice on two large farms. Samples were collected on the day of AI (day 0) and days 5 and 23–24 after AI. The efficacy and practicality of the procedure for assessing the accuracy of heat detection and for early diagnosis of non-pregnancy was studied.

A service to diagnose and manage infertility in dairy animals was operated through the Department of Farm Animal Production and Health of the University of Peradeniya, in conjunction with the training of veterinary undergraduates. The service covered an area in the mid-country and up-country regions of the Central Province comprising of 14 veterinary ranges that had numerous smallholder farms and four large dairy farms. Each veterinary range was visited every three months and each large farm was visited every two months. The veterinarians in charge of the ranges and large farms were requested to identify animals that needed specialist attention (such as those with anoestrus, repeat breeding or uterine infections) and to arrange for their examination during the visits. Each case was subjected to detailed investigations, a diagnosis was arrived at and either treatment was administered to the animals or appropriate advice was given to the owners.

A study was done on repeat-breeding buffaloes to test the efficacy of the treatment regime termed 'Ovsynch', which uses a controlled intrauterine drug releasing (CIDR) device in conjunction with injections of gonadotrophin releasing hormone (GnRH) and prostaglandin $F2\alpha$ ($PGF2\alpha$). Fifteen repeat breeding Murrah buffaloes that had no detectable uterine abnormality on rectal examination were selected. On day 0 of the experiment, each animal was injected with oestradiol benzoate (1 mg) and GnRH (100 µg, Fertagyl), and a CIDR was placed in the anterior vagina. On day 7, they were injected with 500 µg of $PGF2\alpha$ (EstroPlan) and on the following day (day 8) the CIDR were removed. On day 9 a second dose of GnRH (100 µg) was injected and AI was done 14 h after the last injection. All cows were inseminated by one technician using frozen semen. The animals were examined for pregnancy by rectal palpation 60 days after AI.

2.5. Transfer of technology

A series of continuing professional development (CPD) programmes was conducted for veterinarians and AI technicians on AI and infertility management. These were implemented in collaboration with the Institute of Continuing Education in Animal Production and Health (ICEAPH) of the DAPH.

2.6. Introduction of computer databases for data management and monitoring within the national AI service

Three computerized data management packages that were provided by the Joint FAO/IAEA Programme, Vienna, were introduced to the national AI service of the DAPH through demonstration and training activities that were conducted for officers of the Animal Breeding Division, staff of the CAIS, filed veterinarians and AI technicians.

The packages were: (a) Semen Processing Records Management (SPeRM), for recording and managing data at semen processing centres; (b) AIDA Asia, for recording, managing, analysing and reporting data from field AI services; and (c) Livestock Information Management Application (LIMA), for recording and managing data on livestock farms. Action is continuing to provide further technical assistance for their implementation as required.

3. RESULTS

3.1. Performance of AI services at national, regional, district and farm levels

3.1.1. Coverage and performance of the national AI service

The AI service reached only 18.8% of the breedable female cattle population in the country and resulted in the birth of only 6.3% of the calves born annually (Table I). On a regional basis the percentages of breedable cattle served by AI were 68.6% in the wet zone, 16.9 in the intermediate zone and 4.3% in the dry zone [4].

TABLE I. THE COVERAGE AND PERFORMANCE OF THE NATIONAL AI SERVICE IN DIFFERENT AGRO-ECOLOGICAL ZONES OF SRI LANKA DURING 1996

Parameter	Agro-ecological zone			Total
	Wet	Intermediate	Dry	
Total number of breedable cattle	88 836	213 378	278 586	580 800
Number of AI done	60 913	35 989	12 106	109 008
Percentage of breedable cattle bred by AI	68.6	16.9	4.3	18.8
Number of calves born from AI	12 228	4616	1349	18 193
Calving rate from AI (%)	20.1	12.8	11.1	16.7
Total number of calves born	44 418	106 689	139 293	290 400
Percentage of calves born due to AI	27.5	4.3	<0.1	6.3

Source: Department of Animal Production and Health (1997)

3.1.2. Success of AI done in smallholdings

The results are summarized in Table II. The mean interval from calving to first service was 183 ± 87.1 days (n = 211) and the interval from calving to conception was 194 ± 93.9

days (n = 143). The first service conception rate (CR) was 45% and the overall CR was 50.2%, with an average of 1.99 services per conception [7].

3.1.3. *Success of AI done in large farms*

The results are summarized in Table III. The mean intervals from calving to first service and to conception were 111.2 ± 74.2 and 156 ± 92.7 days, respectively. The average first-service and all-service CRs were 50.4% and 53.6%, respectively, with an average of 1.9 services per conception [11].

TABLE II. FERTILITY INDICES IN CATTLE SUBJECTED TO AI ON SMALLHOLDINGS IN FOUR VETERINARY RANGES IN THE MID-COUNTRY WET ZONE

Veterinary Range	Interval from calving (days, mean ± SD)		Conception rate (%):		Services per conception
	First service	Conception	First service	All services	
Gampola	131 ± 51 (n = 34)	113 ± 50.6[a] (n = 17)	50.0 (n = 34)	50.0 (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 ± 81 (n = 84)	200 ± 89.3 (n = 73)	53.6 (n = 84)	60.8 (n = 120)	1.6
Yatinuwara	242 ± 103 (n = 45)	265 ± 131 (n = 15)	33.3 (n = 45)	31.3 (n = 48)	3.2
Overall	183 ± 87 (n = 211)	194 ± 93.9 (n = 143)	45.0 (n = 211)	50.2 (n = 287)	1.99

[a]Based on first-service conceptions only

TABLE III. FERTILITY INDICES OF CATTLE SUBJECTED TO AI IN LARGE FARMS IN THE UP-COUNTRY WET ZONE

Farm	Interval from calving (days, mean ± SD)		Conception rate (%)		Services per conception
	First service	Conception	First service	All services	
Ambewela	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)	60.5 (n = 34)	69.3 (n = 62)	1.4
Dayagama	91 ± 33.5 (n = 35)	148 ± 101.5 (n = 45)	57.1 (n = 35)	60.0 (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

3.2. Evaluation of semen donor bulls and quality control of deep frozen semen

The results from clinical evaluation of semen donor bulls for breeding soundness are summarized in Table IV and the results from examination of semen are given in Table V.

The initial sperm motility in all semen samples studied was within the range of 80-90%, which is fully acceptable. The motility after freezing and thawing was around 60% in semen samples of all except that of one bull (#260), which had a motility of only one half of that in the fresh semen sample.

TABLE IV. CLINICAL EVALUATION OF FIVE SEMEN DONOR BULLS USED IN THE NATIONAL AI SERVICE

Parameter		Identity of bull				
		#260	#261	#262	#263	#264
Body Condition Score (1–5)		3.5	3.5	3.5	3.5	3.5
Body weight (kg)		516	484	441	434	425
General health		Good	Good	Good	Good	Good
Scrotal contents		Normal	Normal	Normal	Normal	Normal
Scrotal circumference (cm)		35	39.5	34.5	36	33.5
Testicular size and shape		Normal	Normal	Normal	Normal	Normal
Left testis	Springiness (0–5)	3	3	3	3	3
	Resilience (0–5)	2	3	3	3	3
	Length (cm)	14.5	16	16	13	13
	Width (cm)	11.5	8	7.5	7	8
Right testis	Springiness (0–5)	3	3	3	3	3
	Resilience (0–5)	2	3	3	3	3
	Length (cm)	14.5	16.6	15.5	14	13
	Width (cm)	11.5	7	8	7	8
Consistency of epididymis		Normal	Normal	Normal	Normal	Normal
Spermatic cord, penis and prepuce		Normal	Normal	Normal	Normal	Normal
Accessory sex glands		Normal	Normal	Normal	Normal	Normal

TABLE V. EVALUATION OF SEMEN FROM FIVE SEMEN DONOR BULLS USED IN THE NATIONAL AI SERVICE

Semen characteristic	Identity of bull				
	#260	#261	#262	#263	#264
Volume (mL)	3.5	4.5	4	6	4
Colour	Milky	Light cream	Milky	Light cream	Milky
Density	DDDD	DDD (D)	DDD	DDD (D)	DDD
Mass motility	+ + + +	+ + + (+)	+ + +	+ + +(+)	+ + +
Sperm concentration (millions/mL)	780	1123	757	1003	632

The results from HOST showed that the sperms of two bulls (#261 and #263) had higher post-thawing membrane integrity than the sperms of the other three bulls.

3.3. Development of intervention protocols for management of repeat breeder cows

The results from the examination of cows subjected to AI for repeat breeding in smallholder farms (n = 1000) and two large state farms (n = 861) are shown in Tables VI and VII, respectively [12]. Categorical analysis of the data from large farms showed that factors related to the cow (breed, body condition score, age, parity, days open, milk yield and previous lactation length) and the farm (herd size, hygienic status of housing and source of labour) influenced the incidence of repeat breeding.

TABLE VI. INCIDENCE OF REPEAT BREEDING AMONG 1000 ARTIFICIALLY BRED CATTLE IN SMALLHOLDER FARMS

Category	Number of Observations	Percentage
1: Less than 3 AI and pregnant or non-pregnant	818	81.8
2: More than 3 AI and pregnant	30	3.0
3: More than 3 AI and non-pregnant (repeat breeders)	152	15.2

TABLE VII. INCIDENCE OF REPEAT BREEDING AMONG 861 ARTIFICIALLY BRED CATTLE IN LARGE FARMS

Category	Number of Observations	Percentage
1: Less than 3 AI and pregnant or non-pregnant	765	89.0
2: More than 3 AI and pregnant	39	4.5
3: More than 3 AI and non-pregnant (repeat breeders)	57	6.6

A total of 100 repeat breeder cows were subjected to clinical examination and microbial culture of swabs collected from the uterus or vagina. Samples from 24 repeat breeder cows that had clinically detectable uterine abnormalities yielded 12 isolates of *Escherichia coli* and two isolates of *Corynebacterium pyogenes*. The organisms isolated from 72 repeat breeder cows that had no detectable clinical abnormalities were *Streptococcus spp.* (10 isolates), *Bacillus subtilis* (14 isolates) and *Peptostreptococcus* (20 isolates).

3.4. Early non-pregnancy diagnosis and infertility management services

The use of milk progesterone for early non-pregnancy diagnosis as a routine procedure on two large farms was abandoned due to several logistical problems. The distance between the farms and the RIA laboratory made transport of samples difficult and assays could not be run on a regular basis due to delays in obtaining shipments of reagents and kits. Preliminary results on the cost of the exercise indicated that it would be too expensive, particularly for smallholder farmers, considering the current low farm-gate price paid for milk.

The services of the mobile fertility clinic were provided regularly to smallholder farmers in the selected veterinary ranges and to the large farms. On average, the number of cases for which assistance was provided each year was 1500 on 60 smallholder farms and

1000 on 15 large farms. The results given above on repeat breeding cows (section 3.3.) are based on some of the cases presented to this fertility clinic.

Treatment of repeat breeding buffaloes with the Ovsynch protocol resulted in a CR of 53% [13]. The cost of hormones for treating one animal was Rupees 1000 (approximately US $ 10).

3.5. Transfer of technology

On the average, three CPD programmes were conducted per year in collaboration with the Institute of Continuing Education in Animal Production and Health of the DAPH. Each programme was of 2–4 days duration and was attended by 12–15 veterinarians. The training consisted of two components: (a) lecture-discussions to update knowledge on topics such as endocrine physiology of reproduction, manipulation of reproduction, infertility management and nutrition-reproduction interactions; (b) half-day practical sessions that provided opportunities for hands-on practice on gynaecological examinations, semen-evaluation, breeding soundness evaluation, use of ultrasound scanning for examining the reproductive organs and use of antibiotics and exogenous hormones in the management of infertility.

To complement these training sessions, a series of publications were produced with financial support from a linked project, the 'SAREC/NARESA Buffalo Research and Development Programme'. These include a 'Handbook for Veterinarians on Cattle and Buffalo Farming', a 'Training Manual for Extension Workers' that provides guidelines and technical information for conducting training for farmers on 10 selected topics, and 10 'Information Leaflets for Farmers' that are used as hand-outs at the farmer training sessions.

3.6. Introduction of computer databases for data management and monitoring within the national AI service

The SPeRM database was installed at the CAIS, Kundasale, and the staff members were trained in its routine use. It is being applied for keeping records on the bulls used for semen collection and for recording the results from semen evaluations done on a routine basis. One of the deficiencies of the application is the lack of a module to keep track of the issues of processed semen from CAIS to the different field stations.

The AIDA Asia package was introduced to the Animal Breeding Division of the DAPH through a workshop that examined the current system of data management, demonstrated the features of AIDA Asia, and discussed its suitability for the purpose. It was found that the existing system of data handling at DAPH, which collects information on AI done in all parts of the country, is slow and has limitations. For example, it is not possible to analyse the results achieved from AI on the basis of each technician or semen donor bull. It was concluded that the AIDA Asia package would be suitable, with the advantages that it would (a) assist in the decentralization of data collection to the provincial level; (b) enable the transmission of data electronically from the provinces to the central level; and (c) provide regular summaries and reports that would give timely feed-back on the performance of AI at the field level. Subsequently, two training workshops were held for the staff members that are responsible for operating the software package. Presently, AIDA Asia is in use at the head office of the DAPH (central level) and efforts are being made by the directorate to expand it to the provincial level.

The LIMA software program was introduced to veterinarians of the DAPH and steps have been taken to install and use it on selected large state livestock farms. It is also currently in routine use for recording and managing data on the University Teaching farm. The experience so far indicates that it is effective for use in medium-sized dairy herds of 30–50 cows.

4. DISCUSSION

The review of national data on AI coverage and its success rate showed that less than 20% of the breedable cattle population is served by AI. When combined with the low calving rate of AI (less than 20%) this means that only about 6% of the calves born per year are due to AI. Overall, it can be concluded that AI has a low impact on the genetic composition of the cattle population in Sri Lanka. Although the coverage rate is high in the wet zone, where infrastructure, farming systems and the delivery mechanism for AI are more developed than in the other two zones, the success rate is still low. As reported by Alexander *et al.* [7], many factors contribute to this low level of performance and efficiency. Ignorance of farmers on the advantages of AI and the proper breeding management of their animals is one aspect. The other includes communication, the resources available to the AI service providers and the motivation of field staff. These result in poor coverage of most areas in the dry and intermediate zones.

The overall success rate of AI in smallholder farms in the mid-country wet zone was in the lower range of acceptability, with high variability in CR between locations. Most farmers in this region resorted to AI for breeding their cows. Since the climate is relatively mild in this region, farmers usually keep animals of European genotypes. Thus they are aware of the value of genetic upgrading through AI for improving milk production. Also, most farmers sell the male calves when they are relatively young, resulting in a scarcity of good bulls for natural service.

In the large farms, which are expected to fulfil a role as multipliers for supply of genetically superior stock to smallholders, the overall success rate of AI was lower than expected. The studies suggest that many factors associated with the chain of events, including the feeding and reproductive management of cows, the quality and handling of semen in the field and the skill of technicians all contributed to poor fertility. Of these, poor heat detection and delays in getting the insemination done stand out as the most important contributory factors.

The study on the five semen donor bulls sampled from those maintained at CAIS, Kundasale, showed that they had good semen characteristics that were fully acceptable. The HOST procedure was introduced to the CAIS for the first time and was well received by the veterinarian in charge of the centre. The HOST positive sperms had intact functioning plasma membranes and ejaculates with high numbers of such sperms are generally expected to yield high fertility.

Previous studies have also reported a similar incidence of repeat breeding in smallholder (18%) and large multiplier (11%) farms [12]. About one third of the repeat breeder animals had detectable abnormalities on clinical examination. Although the reasons for a high incidence of repeat breeding are speculative at this point, the results from the present and previous studies done by our laboratory suggest that poor heat detection, absence of regular culling and inadequacy of veterinary attention on problem cows are possible causes. The large farms have resident veterinarians and the lower incidence of repeat breeders could be due to early diagnosis of problems and appropriate treatment. This study is continuing into a second phase, with more detailed studies aimed at testing the sensitivities of organisms to antibiotics, followed by the conducting of field trails to develop appropriate and cost-effective treatment protocols for managing repeat breeder cows.

The results from the study using the Ovsynch protocol indicates that this practice can be successfully adopted to improve the conception rate in buffaloes that are bred by AI. However, one of the obstacles to promote this treatment to smallholder farmers is its cost. If the reproductive status of the animal is established accurately, the cost of treatment can be reduced by employing different versions of the protocol (e.g. CO-Synch and Select-Synch) that give similar conception rates as the Ovsynch protocol. Further, buffalo milk is sold at a higher price (Rs. 32.00 per litre) compared with cow milk (Rs. 15 per litre) and therefore the cost of treatment can be recovered more quickly from buffaloes than from cows.

The AIDA Asia software was accepted as a useful management tool by the directorate and staff of the national AI service. However, the main problems encountered in entering data at the central level were the large number of records to be handled and the illegibility or incompleteness of many records. The lack of funds to purchase the required hardware has also hindered the implementation of the project. With regard to decentralization and application at the provincial level, the main concern has been the lack of trained personnel, as some users have found the software to be too complex for them to handle. In general, the resources available at the national and provincial levels with regard to hardware and computer literacy have not been compatible with the requirements demanded by the AIDA package.

In order to continue and further develop the activities initiated during this project, the following studies have been planned: (a) continuation of studies on repeat breeder cows so as to develop effective treatment protocols; (b) use of exogenous hormones under field conditions for fertility management of cattle and buffaloes; (c) conduct of routine fertility clinics to assist field veterinary staff; (d) conduct of CPD programmes in collaboration with ICEAPH of DAPH; and (e) consolidation of the application of SPeRM, AIDA Asia and LIMA.

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DEVELOPMENT OF ENZYMEIMMUNOASSAY TEST KITS FOR RAPID QUALITATIVE DETECTION OF PROGESTERONE IN MILK

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Abstract

DEVELOPMENT OF ENZYMEIMMUNOASSAY TEST KITS FOR RAPID QUALITATIVE DETECTION OF PROGESTERONE IN MILK

An enzyme immunoassay (EIA) strip test was developed using lateral flow and dipstick test formats for detection of progesterone in milk of cattle. A variety of membranes were examined as coating materials for the immobilized antibody and the two found suitable were silica gel and Whatman paper. A range of reagent dilutions, volumes and incubation times were studied and for developing the test strip and the optimum conditions were selected. Preliminary studies showed that the test strip is able to differentiate between 0 and 3 nmol/L progesterone within 15 min by producing a colour reaction. However, further studies are necessary to enhance the sensitivity and visual detection ability of the test, determine the most practical format and validate the protocol for routine use in peripheral laboratories and farms.

1. INTRODUCTION

Since the initiation of a ‘Milk drinking promotion campaign’ in Thailand in 1992, the demand for milk and dairy products has increased every year. The present national milk production is 2000 tons/day, equivalent to a value of Baht 25 million/day or Baht 9 billion/year. However, this quantity is insufficient for the national consumption and Thailand has to import dairy products valuing of billions of Baht each year.

As in other countries, artificial insemination (AI) plays an important role in the development of the dairy industry in Thailand. However, follow-up of cows that have been inseminated and assessing success of AI by regular pregnancy diagnosis is a problem. The usual method is by rectal palpation, which can be performed only 2–3 months following AI, and requires an experienced inseminator or veterinarian. These limitations result in long waiting periods before non-pregnant cows are detected, leading to long calving intervals and economic losses to the farmers. Therefore, a technique for early detection of non-pregnancy is required to shorten the interval between an unsuccessful insemination and the subsequent breeding.

Radioimmunoassay (RIA), a technique used in medical applications for over 30 years, has been shown to be applicable for diagnosis of non-pregnancy in cattle by 21 days after AI [1]. In 2001, the Radioisotope Production Program of the Office of Atoms for Peace (OAP) in Thailand initiated a research project titled ‘Development of RIA reagents for milk progesterone measurement in dairy cows’ under the IAEA/RCA project on ‘Improving Animal Productivity and Reproductive Efficiency’. The technique relies on a specific monoclonal antibody in combination with ^{125}I-labelled progesterone as the tracer. The RIA test kit has now been successfully developed and technically validated for analytical use [2]. The technique can be utilized for non-pregnancy testing at 20–22 days after AI with a turn-around time of one week. However, it requires a suitably equipped laboratory with well-trained personnel who are authorized to handle radioactive materials. Hence it is only available in central and regional laboratories and is not widely used.

A follow-up project titled 'Development of a rapid test kit for qualitative detection of milk progesterone' was therefore initiated to solve this problem. The objective was to develop an Enzyme immunoassay (EIA) test kit for qualitative progesterone detection. The technique involves the immobilization of a specific antibody on the capture zone of a selected membrane, followed by the use of a conjugate of progesterone with an enzyme as the tracer, instead of the radioactive tracer that is used in RIA. Since the end point is development of colour change due to enzyme activity, there is no requirement for a costly instrument to make the final reading.

Membrane-based immunoassays generally take one of two forms: (a) flow-through devices where the sample flows down through the membrane, concentrating the sample in a small defined area; or (b) lateral flow devices where the sample is applied at one end of the device and flows laterally through the membrane matrix, binding with secondary and primary antibodies that have been applied on the device matrices during the manufacturing process.

In flow-through assays, the reactants are drawn through the membrane due to its contact with an absorbent bed. First, the analyte and subsequently the tagged secondary antibody are brought into the void volume of the membrane. This dramatically reduces the diffusion time experienced when the reactants in solution must diffuse to the immobilized protein on the surface of the membrane as in a Western blot. This type of assay has the advantage of simplicity both in use and in manufacturing [3–5].

Lateral flow immunoassay systems have recently been developed, which allow for single-step assays that require only sample addition. In these types of assays, the sample is added to one end of the device and moves by capillary action through the interstitial space of the materials in the device. While continuing along this flow path, the sample contacts dried reagents, usually in the form of a tagged secondary antibody, which then migrate with the analyte to a capture zone of membrane-immobilized antibody. Unreacted tagged antibody continues past this capture zone, normally to the end-of-assay indicator.

Another format of diagnostic immunoassays is the 'dipstick' test. This uses a membrane-based detection system as in the lateral flow format mentioned above. However, the volume of analyte is not well controlled, because the strip is placed directly into a container with an excess of the sample that is to be tested.

In the present study the lateral flow and dipstick formats were tested.

2. MATERIALS AND METHODS

2.1. Preparation of samples

Milk samples with and without the addition of sodium azide as a preservative were kindly provided by Dr. Saroch Ngarmkum of the Ratchaburi AI Research Centre, AI Division, Department of Livestock Development, Thailand. The samples were centrifuged at 2700 rpm at 4°C for 30 min. Skim milk was aspirated and stored at 4°C until analysis.

2.2. Selection of membrane

A variety of membranes were tested to select the one with the best performance in immobilizing the specific antibody. Subsequently, an optimal quantity of antibody, sample, antigen-enzyme conjugate and substrate were examined for colour development. The preliminary experiment was performed using milk containing no progesterone as a testing sample for membrane sensitivity.

Briefly, 20 µL of test sample was spotted onto a 1 cm x 1 cm strip of each test membrane material (namely: nitrocellulose, nylon, silica gel and Whatman paper) that had been coated with 20 µL of a diluted specific progesterone antibody, followed by 20 µL of antigen-enzyme conjugate. The reaction mixture was allowed to stand for 60 min at room temperature (25°C), 20 µL of enzyme substrate was added and then allowed to stand for a further 30 min before adding 30 µL of the chromogen. The colour developed was visualized at intervals over a 24 h period. The membranes that gave a distinct colour change within one hour were chosen for further study.

2.3. Optimization of test protocol

In order to get the most sensitive and rapid result, the following tests were performed:

The optimal dilution of progesterone antibody was determined by testing varying dilutions from 1:100 to 1:800. The optimal dilution of progesterone-enzyme conjugate was determined by testing varying dilutions from 1:100 to 1:800. The optimal quantities of antibody and progesterone-enzyme conjugate were tested by spotting 20 µL of test sample onto a 1 cm x 1 cm strip of the membrane coated with the optimal dilution of progesterone antibody in volumes ranging from 5–40 µL, followed by the optimal dilution of antigen-enzyme conjugate in volumes ranging from 5–40 µL.

The optimal sample volume and quantity of enzyme substrate were determined by performing the assay with various volumes of each. Finally, the optimal incubation time for each step of the assay was determined by varying the times and checking the final result.

2.4. Colour differentiation and effect of sodium azide

After selection of the best membrane for immobilizing the antibody and determining the optimal conditions as described above, the ability of the test to produce a detectable colour difference between progesterone concentrations of 0 and 3 nmol/L was examined. These values were selected because the cut-off values used in our laboratory for determining the absence or presence of luteal activity were < 1 nmol/L and > 3 nmol/L, respectively.

The membrane was cut into 0.8 cm x 6 cm strips and all reagents (antibody, antigen-enzyme conjugate, substrate and chromogen) were immobilized at the designated zone. Two methods for testing milk samples were studied: (a) a milk sample was spotted on the marked zone and then colour was developed in a non-carcinogenic solvent (lateral flow format); or (b) a strip was dipped into a milk sample to the marked level and left until colour was developed (dipstick test).

Once the optimal conditions were obtained, the effect of sodium azide (which is used as a preservative for milk samples) on colour development was examined by using samples containing various concentrations of progesterone, with and without sodium azide.

3. RESULTS

3.1. Membrane selection

Of the four types of membrane that were studied, nitrocellulose was found to take too long for colour development (at least overnight), and was therefore not suitable. Although nylon gave the most distinct colour, it was not suitable for the dipstick format, as it was not sufficiently rigid to stand in the sample during the developing process. Silica gel and Whatman paper were comparable in colour development. The cyan colour was visible after standing in the milk sample for 5 min with silica gel and for 10 min with Whatman paper.

3.2. Optimization of test protocol

The optimal conditions that were determined for this test were: 20 μL of 1:100 antibody, 40 μL of milk sample, 25 μL of 1:100 progesterone-enzyme conjugate and 20 μL of enzyme substrate, with or without the addition of chromogen. The incubation time was 5 min with silica gel and 10 min with Whatman paper. The colour visualization should be done after drying for 10 min.

3.3. Colour differentiation and effect of sodium azide

The visible colour that developed within 5 min with silica gel and within 10 min with Whatman paper was able to differentiateble between progesterone concentrations of 0 and 3 nM in milk without sodium azide. However, in the presence of sodium azide, no difference in the colour developed could be observed, even when the progesterone concentration was as high as 10 nM.

4. DISCUSSION

The testing of different membranes revealed that silica gel and Whatman paper were the membranes of choice due to their consistency and rapidity of colour development. Further studies showed that both membranes could differentiate between milk samples containing 0 and 3 nM progesterone, provided sodium azide had not been added. However, Whatman paper is the cheaper of the two and is easier to handle, and will be used in the further development of a rapid test kit in the future.

The optimum reagent combination that was determined for the test strip was: 20 μL of 1:100 progesterone-antibody, 25 μL of 1:100 progesterone-enzyme conjugate and 20 μL of enzyme substrate. The presence of sodium azide interfered with the test and will lead to errors in interpretation of the progesterone concentration. A further problem could be difficulties in interpretation of the test results due differences in the visual ability of individual operators. Therefore, latex technology will be considered during further development of the test, to enhance the colour visualization and reduce potential false negative results.

The strips developed can be used for non-pregnancy testing of cows using milk samples collected without the addition of sodium azide. The method is easy to use and produces a result quickly without the need for advanced equipment. However, it requires further development to improve performance, validate the protocol, establish the shelf-life and compare the results with RIA reference methods and clinical observations.

It is expected that the dipstick test kit will be commercially available soon at a reasonable price, thus making routine application practical and affordable on both large and small cattle farms. It will enable non-pregnant animals to be detected early and repeat AI to be done at the appropriate time, leading to increased production of milk and dairy products in the country.

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DEVELOPMENT OF REAGENTS FOR MEASURING PROGESTERONE IN MILK OF DAIRY COWS BY RADIOIMMUNOASSAY

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Abstract

DEVELOPMENT OF REAGENTS FOR MEASURING PROGESTERONE IN MILK OF DAIRY COWS BY RADIOIMMUNOASSAY

A simple direct radioimmunoassay (RIA) for progesterone measurement in skim milk was developed using a previously raised antiserum against 1-α-hydroxyprogesterone hemisuccinate (progesterone-11-hemisuccinate) and a homologous radioactive label prepared locally by conjugation of 11-α-hydroxyprogesterone hemisuccinate with ^{125}I using the acid anhydride procedure. The RIA is a solid-phase method using antibody coated tubes and incubation for 3 h at 4°C. It was designated the OAP (Office of Atoms for Peace) RIA and its sensitivity, analytical recovery, linearity of response and precision compared favourably with those of the FAO/IAEA 'Self-coating' milk progesterone RIA. Milk samples (n = 71) were assayed using the OAP and FAO/IAEA methods and the results were closely correlated (r = 0.9631). The OAP method was used to monitor the individual progesterone profiles of 10 selected cows and the results were in accordance with the physiological status of the animals.

1. INTRODUCTION

A new management tool available to dairy producers and veterinarians is the simple and rapid measurement of progesterone in milk samples to assess reproductive status of cows. When done in a suitably equipped laboratory, current methods of measurement are accurate, precise and easy to perform, and have high sensitivity.

Progesterone is a hormone produced by the corpus luteum (CL) of the ovary during the oestrus cycle and pregnancy in cattle [1]. It is secreted into the blood and subsequently passes into other body fluids, including milk. Progesterone concentration is at its lowest level at the time of oestrus and reaches its highest level with maturation of the CL during mid-cycle. If the cow does not conceive following breeding, the CL undergoes regression and the progesterone level declines 2–3 days before the next oestrus (Figure 1). If the cow conceives, the CL is maintained, the cow does not return to oestrus and progesterone level remains elevated throughout pregnancy.

Progesterone levels in blood or milk can provide diagnostic information regarding the functioning of the ovary, making it possible to accurately monitor the reproductive status of female cattle [2]. This information is useful in routine reproductive management as well as in diagnosis of low fertility problems. Since milk samples are easier to obtain than blood, they can be used more conveniently for the same diagnostic purposes.

The potential of milk progesterone testing in a reproductive management program are: (a) verification of questionable or suspicious oestrus. (b) pregnancy diagnosis at 21 days after breeding; (c) monitoring postpartum cyclicity. (d) screening for ovarian dysfunction such as cystic and luteinized follicles; and (e) evaluation of the effectiveness of luteolytic agents and infertility treatments.

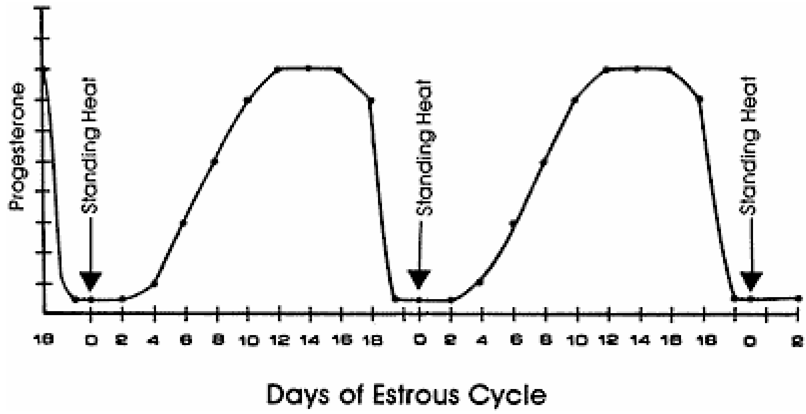

FIG. 1. Cyclic pattern of progesterone in the milk of a cow that is undergoing regular oestrous cycles.

Many methods used to measure progesterone utilize the principle of competitive binding of antigens to a specific antibody. The progesterone in a sample (unknown quantity) competes against a known quantity of progesterone labelled with a suitable 'tracer', to bind with a limited quantity of anti-progesterone antibody. In the procedure for solid-phase radioimmunoassay (RIA), the antibody is coated on the inner surface of assay tubes and the tracer used is progesterone that has been labelled with radioactive Iodine [^{125}I]. The samples to be measured and the tracer are added to the antibody-coated tubes and incubated. The progesterone in the unknown sample competes with progesterone tracer for the binding sites on the antibody. When the unknown sample has high progesterone concentration the binding of competing progesterone tracer is lower, and *vice versa*. After incubation the tubes are decanted and washed, thus removing unbound progesterone and leaving the bound fraction on the wall of the tube. The radioactivity of the bound progesterone tracer is measured with a gamma counter. In addition to the samples, tubes containing a series of standards of known progesterone concentration are included in each assay. These are used for constructing a standard curve, from which the progesterone concentration in each sample is quantified.

The objectives of this study were to produce the reagents necessary for progesterone RIA, optimise the test procedures and technically validate the assay. The locally developed method was designated the OAP (Office of Atoms for Peace) RIA.

2. MATERIAL AND METHODS

2.1. Reagents and chemicals

The monoclonal anti-progesterone antibody (6H11/14), produced in response to progesterone 11-α-hemisuccinyl- bovine serum albumin, was supplied by the Joint FAO/IAEA Programme, Vienna. The progesterone derivative for iodination, 11-α-hydroxyprogesterone hemisuccinate, was obtained from Sigma (United States of America). Radioactive sodium iodide [^{125}I] was obtained from Amersham (United Kingdom).

Histamine, N-methylmorpholine and isobutylchloroformate were obtained from Sigma (USA) and all other reagents and solvents were obtained from BDH chemicals (UK).

2.2. Preparation of assay reagents and coated tubes

The radioligand (progesterone tracer) was prepared according to the protocol recommended by the Joint FAO/IAEA Programme [3]. Briefly, histamine was iodinated with ^{125}I by the chloramine-T method and then coupled to 11-α-hydroxyprogesterone hemisuccinate by the mixed anhydride procedure, as described by Hunter *et al.* [4]. The progesterone-^{125}I histamine derivative was purified by HPLC and diluted with phosphate buffered saline (PBS) before each assay to a final concentration of 25 000–30 000 CPM in 200 μL of solution. The elution pattern of progesterone-11-α-hemisuccinate-2-[^{125}I]-iodohistamine during purification is presented in Figure 2.

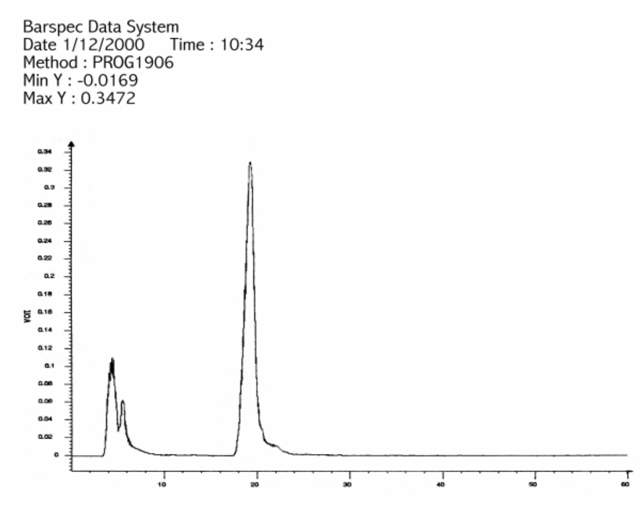

FIG. 2. Elution profile of progesterone-11-α-hemisuccinate-2-[^{125}I]-iodohistamine.

Progesterone standards were prepared by dilution of an ethanolic stock solution of 10 μM of progesterone in skim milk to a final concentration of 1.25, 2.5, 5, 10, 20 and 40 nmol/L [5]. They were calibrated against the reference progesterone standards of the FAO/IAEA, freeze-dried and stored at 4°C.

Antibody coated tubes were prepared by dispensing 300 μL of 1:20 000 dilution of the monoclonal anti-progesterone antibody solution (25 μL of antibody stock solution in 50 mL coating buffer) into 5 ml polystyrene tube (Nunc "Star", immuno quality, 12 x 75 mm) and incubating for 18 h at 4°C. The tubes were decanted and washed twice with washing solution, left to dry at room temperature and stored at 4°C in the presence of a desiccant.

2.3. Comparison of the OAP RIA with the FAO/IAEA reference RIA

The progesterone concentrations in 71 milk samples were determined simultaneously by the FAO/IAEA 'Self-coating' RIA method [6] and the OAP RIA method. The protocol for the latter was as follows:

Fifty microlitres (cf. 40 μL in FAO/IAEA method) of each standard solution (0, 1.25, 2.5, 5, 10, 20 and 40 nmol/L) or milk sample were added into antibody coated tubes, followed immediately by 200 μL of progesterone tracer working solution. The contents of the tubes were mixed and incubated for 3 h (cf. overnight for FAO/IAEA method) at 4°C. The tubes were washed twice with 0.5 mL of washing solution and were counted in a gamma counter for 1 minute. The standard curve was obtained by plotting the percentage of radioactivity (CPM) bound to the tubes containing the standards versus their known progesterone concentration. The percentage of radioactivity bound to the tubes containing each unknown sample was used to determine their progesterone concentration by extrapolation from the standard curve.

2.4. Field validation of the OAP RIA by study of milk progesterone profiles in cattle

The study was undertaken at Nongpho, Ratchaburi, which is situated in the central region of Thailand. Milk samples were collected from cattle on smallholder farms by the Ratchaburi Artificial Insemination Research Centre and delivered to the RIA laboratory at the OAP for testing. The reproductive status of each animal was determined from its history and by clinical examination, and milk samples were collected daily for 60 days. Milk from the morning milking was collected into 30 mL bottles containing one tablet of sodium azide as a preservative, mixed and stored at 4°C until transferred to the laboratory.

In the laboratory, the milk samples were centrifuged at 2000 g and 4°C for 15 min, the fat layer was pierced with a glass rod, the skim milk was transferred to storage vials and stored at 4°C until assayed. The concentration of progesterone was determined by the OAP RIA method.

3. RESULTS AND DISCUSSION

3.1. Validation of reagents and characteristics of the OAP RIA

The progesterone tracer (progesterone-11-α-hemisuccinate-2-[^{125}I]-iodohistamine) produced at OAP was compared with that obtained from Amersham in parallel assays. The results are summarized in Table I and show that both tracers were equally suitable for use in the assay.

TABLE I. CHARACTERISTICS OF PROGESTERONE TRACER PRODUCED AT OAP AND THAT OBTAINED FROM AMERSHAM TO THE MONOCLONAL ANTIBODY IN A ROUTINE ASSAY

Tube contents	Progesterone (nmol/L)	OAP tracer (% B/Bo)	Amersham tracer (% B/Bo)
Standard 1	0	100	100
Standard 2	1.25	79.9	79.6
Standard 3	2.5	66.4	64.9
Standard 4	5	52.5	49.5
Standard 5	10	34.3	30.9
Standard 6	20	27.1	21.6
Standard 7	40	13.6	11.2
Quality Control 1	Unknown	6.3	6.2
Quality Control 2	Unknown	15.9	15.5

The progesterone concentrations in 117 skim milk samples were analysed by reading them against standard curves prepared using the OAP and FAO/IAEA standards. The results were compared by linear regression of OAP standard values on FAO/IAEA values. The relationship gave a slope of 1.0777 with an intercept at 0.2616 nmol/L and a correlation coefficient of 0.9915 (Figure 3). The correlation reflects very good agreement between the two standards.

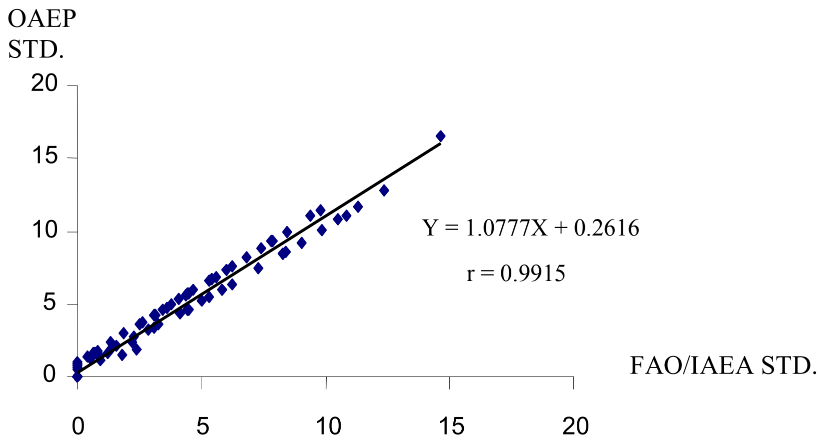

FIG. 3. Correlation between results from milk progesterone concentrations determined using the standards from the OAP RIA and the FAO/IAEA RIA.

The sensitivity of the OAP assay was determined by analysing 20 tubes containing the zero standard in a single assay together with a set of non-zero standards and quality control samples. The sensitivity, defined as the concentration corresponding to the number of counts that are 2.5 standard deviations below the mean was 0.38 nmol/L.

The accuracy was determined by adding known amounts of progesterone to several samples of milk and measuring the concentration. The recovery of added progesterone ranged between 90.6 to 100.8%.

The within-assay precision (reproducibility) was determined by assaying 20 replicates of samples with low, medium and high levels of progesterone in the same assay. The between-assay precision was determined by assaying the same three samples in 10 different assays. The results are presented in Table II.

3.2. Comparison of the OAP RIA with the FAO/IAEA reference RIA

Seventy-one milk samples were assayed simultaneously by the OAP RIA method and the FAO/IAEA RIA method. The regression equation was y = 1.0316x – 0.2161 (r = 0.9631), where y and x are FAO/IAEA and OAEP concentrations, respectively. A comparison of the assay parameters obtained for the two methods is given in Table III.

3.3. Field validation of the OAP RIA by study of milk progesterone profiles in cattle

The progesterone concentrations in serial milk samples collected from 10 cows were used to plot individual progesterone profiles. The diagnosis of reproductive status of each cow based on its progesterone profile and the clinical diagnosis based on the history and rectal palpation of genital organs are given in Table IV. Progesterone profiles illustrating the typical patterns observed in cyclic, pregnant and anoestrous cows are shown in Figures 4–6.

TABLE II. WITHIN-ASSAY AND BETWEEN-ASSAY PRECISION FOR THE OAP RIA METHOD

Parameter	Number of replicates or determinations	Mean Value (nmol/L)	Coefficient of Variation (%)
Within-assay precision			
Sample 1	20	2.5	12.7
Sample 2	20	6.6	5.4
Sample 3	20	18.2	5.8
Between-assay precision			
Sample 1	10	2.7	8.0
Sample 2	10	4.9	12.3
Sample 3	10	21.4	10.9

TABLE III. COMPARISON OF ASSAY PARAMETERS FOR THE OAP RIA AND FAO/IAEA RIA METHODS

Parameter	OAP RIA	FAO/IAEA RIA
Total counts (CPM)	27 540	27 685
Maximum binding (%)	40.5	44.9
Correlation coefficient	0.9955	0.9973
Sensitivity (nmol/L)	0.38	0.17
Within-assay precision (%)	5.4–12.7	8.8–10.0
Between-assay precision (%)	8.0–12.3	9.3–11.1
Accuracy (recovery, %)	90.6–100.8	–

TABLE IV. CORRELATION BETWEEN THE DIAGNOSIS OF REPRODUCTIVE STATUS BASED ON PROGESTERONE PROFILES AND CLINICAL EXAMINATION

Cow No.	Progesterone (P4) profile and diagnosis	Clinical diagnosis
1	P4 maintained above 3 nmol/L; Pregnant	Pregnant
2	P4 maintained above 3 nmol/L; Pregnant	Pregnant
3	P4 maintained above 3 nmol/L; Pregnant	Pregnant
4	P4 maintained above 3 nmol/L; Pregnant	Pregnant
5	P4 maintained above 3 nmol/L; Pregnant	Pregnant
6	P4 fluctuating; Cyclic cow	Non-pregnant, returned to heat
7	P4 fluctuating; Cyclic cow	Non-pregnant, returned to heat
8	P4 fluctuating; Cyclic cow	Non-pregnant, returned to heat
9	P4 basal; Non-cyclic cow	Postpartum anoestrus
10	P4 basal; Non-cyclic cow	Postpartum anoestrus

4. CONCLUSION

The results show that the RIA method developed satisfies the standard criteria of accuracy, precision, sensitivity and specificity for the determination of progesterone in milk samples. Its introduction has led to increased laboratory efficiency and faster service than with the original method, as the total assay time is 4–5 h in comparison to 24 h. Therefore, the results could be returned to the farmers in a shorter period.

The interpretation of the reproductive status of 10 selected cows on the basis of progesterone profiles determined by the OAP method corresponded well with the clinical diagnosis based on history and rectal palpation of reproductive organs. Thus it can be concluded that the method is suitable for field use in dairy cattle.

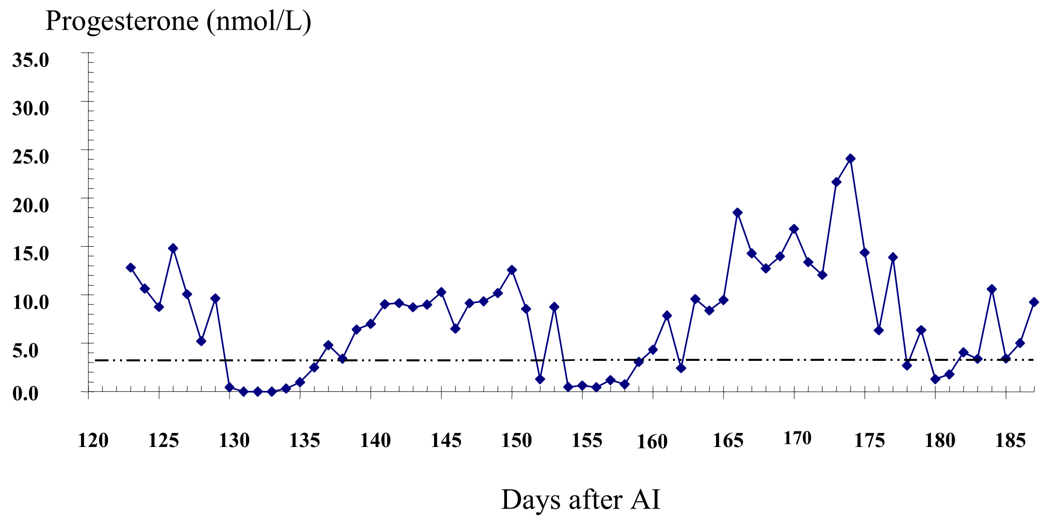

FIG. 4. Progesterone profile in a cyclic cow obtained from measurements using the OAP RIA.

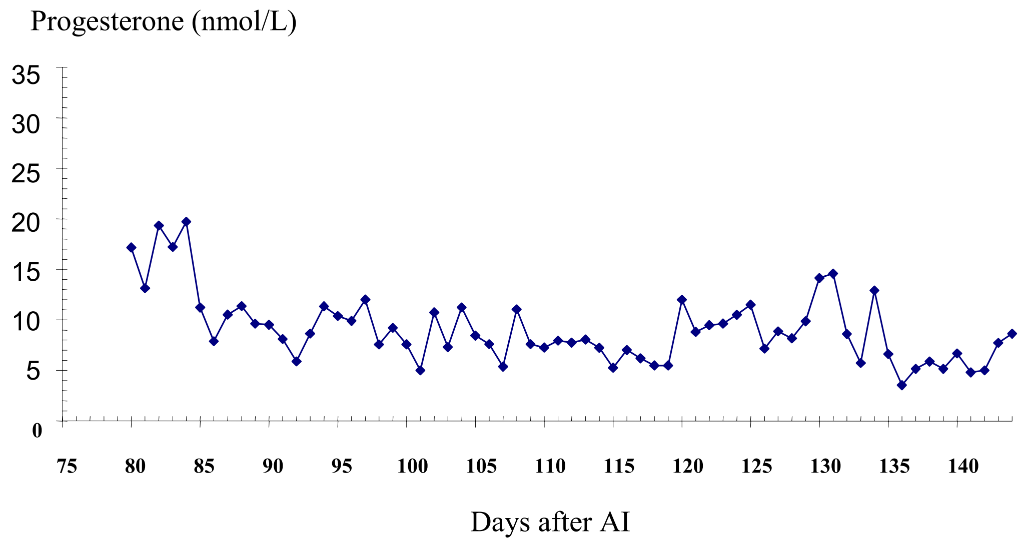

FIG. 5. Progesterone profile in a pregnant cow obtained from measurements using the OAP RIA.

On-farm milk progesterone testing can be a useful tool to both dairy producers and practicing veterinarians. The benefits of simple, quick and accurate tests for determining milk progesterone levels are obvious. However, they will only supplement, not replace, a complete herd health program that includes regular examinations by a veterinarian as well as good oestrus detection and accurate record keeping. The application of milk progesterone testing in various aspects such as verifying suspected oestrus, identifying non-pregnant cows 21 days after breeding and monitoring treatments administered to problem cows, can help reduce long calving intervals. Milk progesterone testing is a management aid that, when used judiciously, has the potential to significantly improve the reproductive efficiency and profitability of most dairy farms.

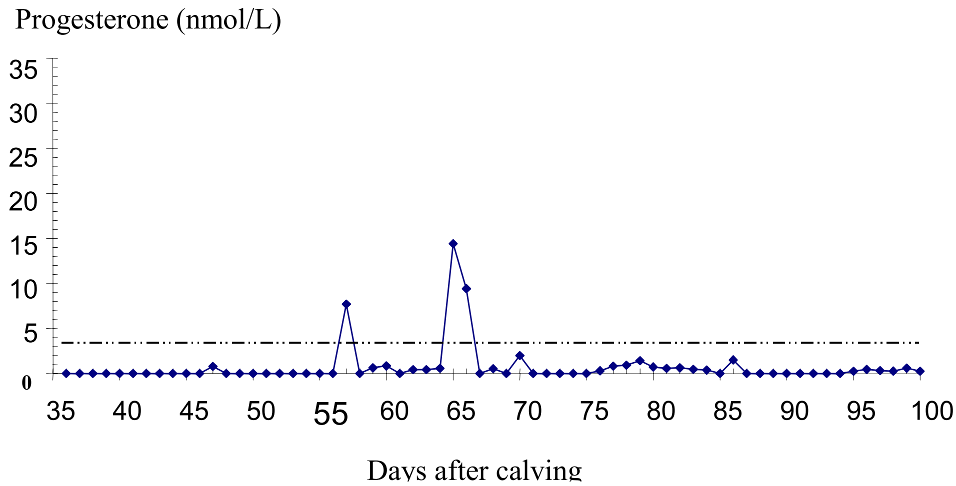

FIG. 6. Progesterone profile in an anoestrous cow obtained from measurements using the OAP RIA.

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