IAEA-TECDOC-1571

Application of Radioimmunoassay in Improving the Reproductive Management of Smallholder Dairy Cattle

Results from an IAEA Regional Technical Cooperation Project in Africa

Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

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FOREWORD

Improvement of livestock production in the African region has been embarked on in many countries with support from their own resources as well as from external donors. Although artificial insemination (AI) has been applied for cattle breeding for many years in Africa as a means of accelerated genetic improvement of the indigenous stock, the overall productivity of this sector has continued to be low. Some of the factors that contribute to the poor output include inadequate management practices, poor nutrition, occurrence of reproductive disorders, systemic diseases and parasites.

The International Atomic Energy Agency (IAEA) has taken a keen interest in supporting efforts to improve livestock production in Africa through national and regional technical cooperation projects. In the recent past, two successive regional projects were implemented under the framework of the African Regional Cooperative Agreement (AFRA) programme. The first was entitled Development and Field Evaluation of Animal Feed Supplementation Packages and had two main components: (a) the development and dissemination of cost-effective and sustainable feed supplementation packages which are based on locally available feed resources; and (b) establishment of the 'self-coating' Radioimmunoassay (RIA) technique for measuring progesterone in the milk and blood of ruminants. The second was entitled Increasing and Improving Milk and Meat Production and had the objectives of: (a) assessing and improving AI programmes for small-scale dairy farmers; (b) establishing sustainable early non-pregnancy diagnosis (N-PD) and related services based on RIA; and (c) harmonizing managerial and field practices within the region.

Some of the issues, among others, which have been identified through the above projects as being responsible for low productivity of cattle in Africa include: (a) inadequate follow-up of offspring arising from AI (e.g. poor calf management leading to retarded growth or even death, and failure to record the performance of improved genotypes); (b) lack of appropriate selection criteria for breeding stock for improved productivity; (c) inadequate management practices coupled with poor nutrition and absence of disease control measures; and (d) lack of regular programmes for training and continued education for extension workers and farmers.

This publication contains the results obtained by Member States in the project activities of Increasing and Improving Milk and Meat Production. 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 for this publication was P. Boettcher of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. He was assisted by O. Perera (Sri Lanka) in the compilation of this publication.

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IMPROVING THE REPRODUCTIVE MANAGEMENT OF SMALLHOLDER DAIRY CATTLE AND THE EFFECTIVENESS OF ARTIFICIAL INSEMINATION SERVICES USING AN INTEGRATED APPROACH: A SUMMARY

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1. INTRODUCTION

Economic development is steadily progressing in Africa. 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 (MS) of the African Regional Cooperative Agreement (AFRA) 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 used in many African 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 RAF/5/046 was initiated during the 1999–2000 biennium with the long-term objective to increase productivity and profitability in the production of milk and meat in AFRA MS. In the short term, the objectives were:

- assessing the performance of existing AI programmes for small-scale dairy farmers and identifying constraints,
- formulating and assisting in the implementation of remedial measures including appropriate strategies,
- establishing sustainable routine non-pregnancy diagnosis (N-PD) and related services to farmers, and
- harmonizing managerial and field practices and sharing of expertise within the region.

Due to the long-term nature of the project objectives and the desire to promote sustainability, the project was twice extended for two additional two-year periods until the 2003–2004 bienium. This extension allowed the consolidation of the results obtained by the project and encouraged broader transfer of the outputs to stakeholders. Throughout the course of the project, 17 MS participated to some degree: Algeria, Burkina Faso, Egypt, Ethiopia, Ghana, Kenya, Mali, Morocco, Senegal, South Africa, Sudan, Tanzania, Tunisia, Uganda, Zambia and Zimbabwe. For various reasons, some MS did not participate for the full duration of the project.

Each participating MS nominated a Project Co-ordinator (PC). The project commenced with an initial Project Co-ordination meeting in Rabat, Morocco in May 1999. The main purposes of this meeting were to plan project activities and to train PCs on the use of the Artificial Insemination Database Application (AIDA) for recording, analyzing and interpreting field and laboratory data. Subsequent meetings for project review and planning took place in Sidi Thabet, Tunisia in October 2000, and in Addis Ababa, Ethiopia in January 2002.

In addition, the following interim meetings were held periodically to address particular issues of concern to the project:

- A Task Force meeting on “Training of Artificial Insemination (AI) technicians, field assessment of fertility and database management” was held 21–27 November, 1999 in Pretoria, South Africa;
- A meeting to review the results on using AIDA was held 11–17 June, 2000 in Entebbe, Uganda;
- A Task Force meeting to “Harmonize procedures for selection and management of AI bulls and use of semen technology in African countries”, was held 7–11 May, 2001 in Arusha, United Republic of Tanzania,

- A Task force Meeting on “Customisation of AIDA for African conditions”, was held 13–17 May 2002 in Nakuru, Kenya

- A Task force meeting on “Cost-benefit analysis, cost recovery and regional production of RIA reagents” was held 17–21 February 2003 in Dakar, Senegal, and

- A Task force meeting to “Review results from cost-benefit analyses and regional supply of progesterone tracer, and finalize cost-recovery strategy” was held 1–5 March, 2004, in Irene, South Africa

The following Regional Training Workshop was also organized under the framework of the project:

- Training workshop on “Regional (AFRA) Training Workshop to train trainers on improved breeding technologies and integration of progesterone-based farmer services into AI systems”, 7–11 October 2002, Rabat, Morocco.

The nuclear techniques addressed in the above workshops included the use of 125I-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 N-PD in cattle.

The final meeting was held 4–8 October 2004 in Ouagadougou, Burkin Faso. 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 an Agency TECDOC.

This TECDOC contains papers summarizing the work done in eight of the participating MS. 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) the use of the AIDA database for organization of information on animal reproduction; d) epidemiology of factors affecting reproductive efficiency at the farm and cow level; e) interventions designed to improve productivity; f) synchronization of oestrus; and g) cost-benefit analysis of various reproductive strategies. Table I shows which countries reported on the different activities.

TABLE I. SUBJECTS ADDRESSED IN PAPERS FROM EACH PARTICIPATING COUNTRY

Activity	BKF	ETH	KEN	SAF	SEN	SUD	TUN	ZAM
Field surveys	X	X	X		X		X	X
Radioimmunoassay	X	X	X	X		X	X	X
Databases	X	X	X				X	X
Epidemiology	X	X	X				X	X
Interventions			X		X	X		
Oestrus synchronization	X					X		
Economic analysis	X	X			X			

BKF = Burkina Faso, ETH = Ethiopia, KEN = Kenya, SAF = South Africa, SEN = Senegal, SUD = Sudan, TUN = Tunisia, ZAM = Zambia

3. SYNTHESIS OF RESULTS AND MAJOR ACHIEVEMENTS OF THE PROJECT

3.1. Infrastructure and human resources

- Functional RIA labs have been established in all participating MS, including decentralized mini-RIA labs in some (Table II).

- Most labs have obtained certification from national authorities where necessary.

- Production and supply of 125I-progesterone tracer has been established within the region in South Africa and production of standards and Internal Quality Control (IQC) samples have been initiated in all MS.

- Potential for procurement of most required consumables (except Monoclonal Antibody) exists within the region.

TABLE II. STATUS OF RADIOIMMUNOASSAY (RIA) LABORATORIES IN EACH PARTICIPATING COUNTRY

Country	Total RIA Labs	Total RIA Technicians	Gamma Counter
Burkina Faso	1	2	1-well
Ethiopia	1	1	1-well
Kenya	2	6	1-well
South Africa	1	1	12-well
Senegal	1	2	8-well
Sudan	2	5	12-well
Tanzania	1	4	1-well
Tunisia	1	2	8-well
Uganda	1	4	1-well
Zambia	1	2	1-well

3.2. AI services

Tables III and IV provide some basic statistics about the AI services offered in each country.

- The RIA technology has offered a complimentary method for providing non-pregnancy and infertility diagnostic services to livestock farmers and has resulted in better knowledge on performance and constraints of AI in participating MS.

- AI services have been strengthened through better training of AI technicians using standardized guidelines and distance learning (ICT) materials.

- Procedures for selection and management of bulls at AI centres have been improved and methods for semen processing and handling have been standardized.

TABLE III. NUMBERS OF INSEMINATIONS AND COSTS OF SEMEN IN EACH COUNTRY

	No. of AI per year		Semen cost ($/dose)	
Country	Currently	In 5 years (expected)	National	Imported
Burkina Faso	500	3 000	7	20
Ethiopia	60 000	300 000	0.50	5
Kenya	300 000	600 000	4	8
S. Africa	600 000	600 000	7.50	10
Sudan	Few	50 000	1	0.25[a]
Tanzania	20 000	100 000	1	5
Tunisia	238 000	300 000	2	2–50
Uganda	30 000	50 000	4	5
Zambia	5 000	10 000	n/a	6–10

[a]Farmer pays for transport costs only, due to a programme of subsidies from the government.

TABLE IV. INFORMATION ABOUT ARTIFICIAL INSEMINATION SERVICES AT THE FARM LEVEL IN EACH COUNTRY

Country	Fee per AI ($)	Trend of AIs	Bonus for AIT[a] based on	Pregnancy diagnosis done by
Burkina Faso	30 or 70[b]	Increasing	Each AI	Vet
Ethiopia	0.50	Increasing	None	Vet or AIT
Kenya	8	Increasing	None	AIT
S. Africa	15	Constant	Each AI	Vet or AIT
Sudan	2	Increasing	Tips	Vet or AIT
Tanzania	5	Increasing	Each AI	Vet or AIT
Tunisia	10–60	Increasing	Varies	Vet
Uganda	8	Increasing	Each AI	Vet or AIT
Zambia	8	Increasing	None	Vet or AIT

[a]AIT = AI technician; [b]with oestrus synchronization

3.3. Data management and economic analyses

- A software application for AI data management (AIDA-Africa) has been developed and validated for use under African conditions and personnel from national AI services have been trained.

- A second application for Semen Processing Records Management (SPeRM), developed under a related project in Asia, has been adopted in several African MS.

- There is enhanced knowledge on cost-benefit analyses, impact assessment, monitoring and evaluation in the participating MS.

3.4. Livestock extension services and farmers

- Closer dialogue has been established between project personnel, extension services and farmers.

- All MS have conducted in-country training, education and sensitization activities for farmers, AI personnel and policy makers. These comprised some 220 activities, involving over 4 300 participants, and amounted to more than 260 000 man-days (TABLE V).

- Farmers now appreciate the role of project personnel in assisting them to identify constraints and to institute improved management practices.

3.5. Collateral benefits

- Project personnel have become more competent and confident in the use of nuclear and related techniques in livestock production. They have received greater professional recognition and appreciation in their institutes and countries.

- There has been sensitization of decision makers on the role and proper application of AI in livestock development.

- The image of the IAEA has been enhanced through greater awareness of the public and policy makers on the peaceful uses of nuclear technology for increasing livestock production.

- Enhanced communication, interaction and collaboration in livestock production now exist between participating MS. This has resulted in greater availability and exchange of expertise within the region.

- The facilities established through the project have enhanced capability for undergraduate and post-graduate training in many Universities and research institutes. This has resulted in greater numbers of livestock professionals with knowledge on applications of nuclear techniques.

- The facilities established have been used for wider studies, including a better understanding of the reproductive performance of local breeds of livestock and wildlife.

4. CONCLUSIONS

The work done during the course of project RAF/5/046 has helped to expand the use of AI and increase its efficiency. However, several key problems were identified with regard to reproduction of livestock. 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 timing of AI; and uterine infections.

In addition, other issues which have been identified as contributing to low productivity of cattle in Africa include:

- Inadequate follow-up of offspring arising from AI (e.g. poor calf management leading to retarded growth or even death, and failure to record the performance of improved genotypes);

- Lack of appropriate selection criteria for breeding stock for improved productivity;

- Inadequate management practices coupled with poor nutrition and absence of disease control measures;

- Lack of regular programmes for training and continued education for extension workers and farmers.

- Based on these observations it can be concluded that a necessity exists to ensure that the genetically improved calves that are being born are properly managed, fed and utilized in order to derive the optimum benefits for increasing milk and meat production in the African region

TABLE V. SUMMARY OF TRAINING ACTIVITIES CONDUCTED BY PARTICPATING COUNTRIES

Country	Activity	Target	No. of activities (N)	Total No. of participants	Total No. of days
Burkina Faso	Sensitization	Farmers	3	50	3
	Sensitization	Decision makers	1	11	1
	Seminars	AITs[a]	2	11	2
	Training	RIA, AIDA	2	7	10
	Graduate Thesis	Students	1	1	300
	Diploma	Technologists	3	3	150
Ethiopia	Sensitization	Farmers	2	104	2
	Seminars	Livestock professionals	3	31	3
	Training	AITs	2	24	2
Kenya	Workshops	Vets, AITs, Farmers	2	120	4
	Training	RIA lab	2	12	15
	Field days	Farmers	3	300	3
	Scientific Visits	IAEA Fellows	1	1	14
	Diploma	Lab technologists	3	5	150
	Post-graduate	MSc	1	1	365
Tunisia	Seminars	Vets, farmers	5	150	5
	Training	AITs, AI, RIA	17	230	400
	Fellowships	IAEA Fellows	9	9	230
	Graduate Thesis	Vet students	2	2	365
	Post-Graduate	DVM	3	3	730
South Africa	Sensitization	Managers	1	4	1
	Training	AI, RIA, Fellows	38	309	190
	Field days	Farmers	15	200	15
	Post-graduate	Diploma	1	1	365
Sudan	Training	AI, RIA	7	65	35
	Seminar	Ultrasound	1	20	1
	Post-graduate	PhD	2	2	1095
Uganda	Sensitization	Farmers	16	600	32
	Training	AI, RIA	11	166	180
	Fellowships	IAEA Fellows	2	6	74
Tanzania	Training	AI, RIA	14	172	386
	Seminar	AITs, vets	1	20	1
	Sensitization	Farmers	30	1500	30
Zambia	Sensitization	Farmers	1	10	1
	Training	AI, AIDA, RIA	3	15	10
	Post-graduate	Diploma	2	4	60
	Fellowships	IAEA Fellows	1	1	60
	Field days	Farmers, others	1	200	2

[a]AITs = AI technicians, AIDA = Artificial insemination database application, RIA = radioimmunoassay

THE USE OF RADIOIMMUNOASSAY TECHNIQUE FOR EARLY DIAGNOSIS OF NON-PREGNANCY AND ESTABLISHMENT OF REPRODUCTIVE PERFORMANCE OF CATTLE IN BURKINA FASO

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Abstract

A survey was conducted in 14 farms, mainly in the peri-urban areas of Ouagadougou. A total of 407 artificial inseminations (AI) done by six technicians were monitored, comprising 106 done after natural oestrus and 301 done after synchronization of oestrus by use of a progestagen ear implant. Samples of milk or plasma (n=1132) were collected on days 0, 10–12 and 21–24 after AI and progesterone was measured by radioimmunoassay (RIA). The results from the three samples showed that only 26% of the animals conceived and 42.6% were anoestrous or acyclic at the time of AI. The overall conception rate (OCR) was 31.4%. It was significantly higher ($p < 0.005$) when AI was done after a natural oestrus (51.8%) than after a synchronized oestrus (26.2%), with the mean number of services per conception being 1.9 and 3.8 for these two groups, respectively. The OCR was significantly higher ($p < 0.01$) in crossbreds (52%) than in Zebus (31.2%) or local taurines (28%). Overall, the mean intervals from calving to first service and calving to conception were 141.1 ± 62.3 days and 145.6 ± 57.7 days. The interval from calving to conception was influenced by the system of feeding on different types of farms, being significantly longer (180.4 ± 79.3 days) in cows provided grazing only, compared with those provided grazing and supplementary feeding (134.6 ± 44.4 days, $p < 0.05$). This interval was shorter in crossbreds (116 ± 32.4 days) and Zebus (122.4 ± 40.6 days) than that in local taurines (169.5 ± 59.6 days), but this difference was not significant. Calvings from December to February were followed by shorter intervals. The level of education of AI technicians had a significant effect ($p < 0.03$) on OCR, but the length of experience had no effect. Self employed technicians achieved a higher OCR (39.2%) in comparison to government technicians (29.6%). The cost of assaying one milk sample for progesterone was estimated to be €3.14. The revenue forgone when a cow fails to conceive to an AI, resulting in an unproductive period of one cycle length (i.e. 24 extra days open), was estimated to be €47.36 in extensive farms and €100.64 in intensive farms. It was concluded that monitoring reproduction through RIA of progesterone was cost-effective in improving reproductive efficiency.

1. INTRODUCTION

In Burkina Faso the cattle population is estimated to be 7 311 544 head [1]. Livestock contributes 11% to the national economy and represents the second-ranked export product after cotton. In spite of the large number of cattle, Burkina Faso still imports milk and its by-products, valuing around €17 million annually. The contribution of local production is not commensurate with the number of cattle due to poor productivity. National production is estimated to be 172 000 tons of milk, whereas the need for the human population is 500 000 tons. Therefore, attempts are being made to tackle this problem by improving the productivity of the local breed through selection and crossbreeding using artificial insemination (AI) and increasing the reproductive performance.

In Burkina Faso, around 30 AI technicians have been trained, but only 12 (both public and private) are regularly performing AI. Semen of the Montbéliard, Tarentais, Brown Swiss, Holstein, Jersey and Belgian Blue-White has been imported, while that of Azawak, Gir and Girolando are produced locally. The use of AI is still low, with fewer than 5000 inseminations done per year and restricted mainly to peri-urban farms. Most of the inseminations are done after synchronization of oestrus, which is expensive, costing around €53.40 per cow.

The study described here was undertaken within the framework of the AFRA project RAF/5/046 for improving milk and meat production through (a) assessing the performance of AI programmes for small-scale dairy farmers with the identification of constraints; (b) formulating and assisting in the implementation of remedial measures using appropriate strategies; (c) establishing sustainable routine non-pregnancy diagnosis (N-PD) and related services to farmers; and (d) harmonizing managerial and field practices and sharing expertise within the region. At the national level the specific objectives were mainly to improve AI services and to provide early N-PD services to farmers in order to enhance reproductive efficiency by detecting reproductive disorders and establishing factors related to conception rates. Field and laboratory activities were started in June 2002.

2. MATERIALS AND METHODS

2.1. Farms and animals

Initially, discussions were held with the staff of the collaborating institutes, public and private AI technicians and farmers who were already using the technique of AI, to provide them with information on the planned activities and to ensure their active participation.

Fourteen farms (3 government and 11 private) were included in the study. The majority of the farms were located in the peri-urban area around Ouagadougou but some were 200 to 400 km away. The farms were all dual-purpose, with dairy production being the main objective. There were three farming systems with the following characteristics: (a) government farms: large herds with good management, feeding and health care; AI is done on some cows and all farm data are recorded in a computer; (b) Peri-urban system: small herds with improved breeds of cattle derived through importation of sires, dams or frozen semen; feeding is good, based on grazing, roughage, concentrate and supplementation and the majority are focused on dairy production; breeding is controlled and recording of data is done on cards; (c) Rural system: local breeds of cattle of low productivity, kept under poor management, housing and health care with extensive grazing and no supplementary feeding; no AI is done and no records are maintained.

The breeds of cows included the study were local Zebus (Azawak, Goudali and Zebu Peulh), local taurines (Lagunaire and Borgou of Benin) and crossbreds between the local breeds and imported taurines (Montbéliard, Holstein, Jersey and Tarentais). The parity of cows ranged from 0 to 8 and bulls were present in all the farms. Milking was done manually and lactation length ranged from 5 to 9 months.

2.2. Collection of data

Six AI technicians with adequate experience participated in the study. A total of 407 inseminations were monitored, which comprised 106 done after natural oestrus and 301 done after synchronization of oestrus using an ear implant containing a progestagen (Norgestomet, 'CrestarR'). The implant was kept in place for 10–11 days, an injection of prostaglandin analogue (Cloprostenol, 0.5 mg) was given two days before removing the implant and an injection of Pregnant Mare Serum Gonadotrophin (PMSG, 250 i.u.) was given at the time of removal. Each cow was inseminated twice, at 48 and 72 hours after implant removal.

Data related to farms, AI technicians, semen batch and cows were recorded and entered in to the Artificial Insemination Database Application (AIDA) provided by the Joint FAO/IAEA Programme on Nuclear Techniques in Agriculture, Vienna [2].

2.3. Collection and processing of samples

Samples of milk (in lactating cows) or blood (in dry cows and heifers) were collected on the day of AI (day 0) and on days 10–12 and 21–24 after AI.

For milk samples, vials containing sodium azide preservative were given to AI technicians. After collection, the vials were labelled and returned to the laboratory within a few days. On arrival they were centrifuged at 2000 *g* for 15 minutes and cooled to 4°C to harden the fat layer. The skim milk was extracted and stored deep frozen at -20°C until assay.

For plasma samples, blood was taken from the jugular vein using tubes containing heparin as anticoagulant. After collection, blood samples were immediately stored at 4°C in an ice bath. They were centrifuged within 2 to 4 hours of collection at 2000 *g* for 20 minutes. Then plasma was drawn off, transferred to storage vials and deep frozen at -20°C until assay.

2.4. Radioimmunoassay of progesterone

Progesterone concentration was determined in plasma and skim milk by radioimmunoassay (RIA) using two methods. Plasma samples were assayed by the 'Pre-coated' tube method [3] and milk samples were assayed by the 'Self-coating' method [4], using kits and protocols provided by the Joint FAO/IAEA Programme on Nuclear Techniques in Agriculture, Vienna. Each sample was assayed in duplicate, together with a series of standards and two control samples. Radioactivity was measured using a single well gamma counter.

2.5. Benefit-cost analysis (BCA) and impact assessment

To estimate the economic loss (or revenue forgone) from an unproductive cow due to the open (non-pregnant) period being longer than the optimum period, we used the following parameters and assumptions:

- Value of milk loss per day, based on a mean milk production per day of 2–3 litres for local breeds and 8–12 litres for crossbreds;

- Value of calf loss per day, based on a mean first calving age of 38 months for local breeds and 30 months for crossbreds, a calving interval of 14 months for extensive farms and 12–13 months for intensive farms, and a life-time production of 7–8 calves);

- Monetary value of a new born calf, based on an estimation of value of a weaned calf, less the feed and management costs;

Calculation of the revenue forgone was done based on the assumption that one missed oestrous period would lengthen the days open by 24 days (one cycle length). This included the loss occurring due to the cow not producing milk for one unproductive cycle plus the loss due to the proportional value of a calf for that period.

For further analysis of benefits and costs, a questionnaire survey was done on the farmers and milk traders. Impact assessment was done through discussions and interviews with end-users of the N-PD technique, including farmers, AI technicians and policy makers.

2.6. Data analysis

All the data collected from farms and the RIA laboratory were entered into the AIDA software application [2]. Summary tables were generated and the data were exported in the form of MS Excel files for further analysis using SPSS and Epi Info. The main parameters analysed were the intervals from calving to first service and conception, conception rate, mean services per conception and the factors affecting these parameters in relation to farm, cow, semen batch and AI technician. Comparisons were done using Student's Test or One way ANOVA.

3. RESULTS

3.1. Progesterone concentration in milk and blood samples

During the two years, 336 animals (cows and heifers) were inseminated, some more than once. There were 301 AI done after synchronization and 106 AI after natural oestrus. A total of 1132 samples of milk and plasma were collected, out of which 917 samples were assayed by RIA. The results from samples collected on the day of AI (day 0) are given in Table I and show that 75.6% of AIs were done when progesterone was low, which indicates that there was no corpus luteum (CL) and the animal could have been in oestrus or anoestrus.

TABLE I. PROGESTERONE CONCENTRATION ON THE DAY OF ARTIFICIAL INSEMINATION (DAY 0) AND THEIR INTERPRETATION

Progesterone	Number of animals	%	Interpretation
Low[a]	239	75.6	AI done at a time other than the luteal phase (i.e. probably during oestrus or anoestrus)
High[b]	45	14.2	AI done during the luteal phase or pregnancy
Intermediate[c]	32	10.1	AI done either too early or too late relative to oestrus, or result due to assay variability

[a]Low: <1 nmol/L/, [b]High: >3 nmol/L, [c] Intermediate: 1–3 nmol/L

The results from samples collected on day 0 and 10–12 days later are given in Table II. They show that only 35.5% of the cows had a low progesterone value at AI followed by a high value 10–12 days later, indicating ovulation and development of a CL, while 41.5% of the cows had two successive low values, indicating that they were anoestrous or acyclic.

TABLE II. PROGESTERONE CONCENTRATION ON THE DAY OF ARTIFICIAL INSEMINATION (DAY 0) AND 10–12 DAYS LATER

Day 0	Day 10–12	Number	%	Interpretation
Low[a]	High[b]	94	35.5	AI during an ovulatory cycle
Low	Low	110	41.5	Anoestrus, anovulation or short luteal phase
High	High	14	5.3	AI on pregnant animal or luteal cyst
High	Low	5	1.9	AI done during luteal phase
*	*	42	15.8	*At least one sample had an intermediate[c] value; other clinical data is required for interpretation

[a]Low: <1 nmol/L/, [b]High: >3 nmol/L, [c] Intermediate: 1–3 nmol/L

The results from all three samples and the findings from pregnancy diagnosis done by rectal palpation are given in Table III. They show that only 26% of the animals conceived to AI. The mean progesterone concentrations in the three successive samples from cows that conceived to the AI are shown in Figure 1.

TABLE III. PROGESTERONE CONCENTRATION ON THE DAY OF ARTIFICIAL INSEMINATION (DAY 0), 10–12 AND 21–24 DAYS LATER, TOGETHER WITH RESULTS FROM MANUAL PREGNANCY DIAGNOSIS (PD)

Day 0	Day 10–12	Day 21–24	PD	Number	%	Interpretation
Low[a]	High[b]	High	+	65	26.1	AI during an ovulatory cycle, pregnant
Low	Low	Low	–	106	42.6	AI during anoestrus or anovulatory cycle, unsuccessful AI
Low	Low	High	+	11	4.4	Animal pregnant but not by AI
High	High	Low	–	7	2.8	AI on pregnant animal, embryonic mortality
High	High	High	+	6	2.4	AI on pregnant animal, pregnancy maintained
High	Low	High	±	2	0.8	AI during luteal phase, some animals pregnant but not by AI
*	*	*	±	62	24.9	At least one sample had an intermediate[c] value; other clinical data is required for interpretation

[a]Low: <1 nmol/L/, [b]High: >3 nmol/L, [c] Intermediate: 1–3 nmol/L

3.2. Reproductive performance of cows

The summary of reproductive performance in animals inseminated after a natural or synchronized oestrus is shown in Table IV. Overall, the mean intervals from calving to first service and calving to conception were 141.1 ± 62.3 days and 145.6 ± 57.7 days, respectively. These two intervals were similar because many cows that did not conceive to the first AI could not be followed until subsequent conception. The interval to conception was shorter in cows subjected to AI after natural oestrus than in those that were synchronized ($p < 0.05$).

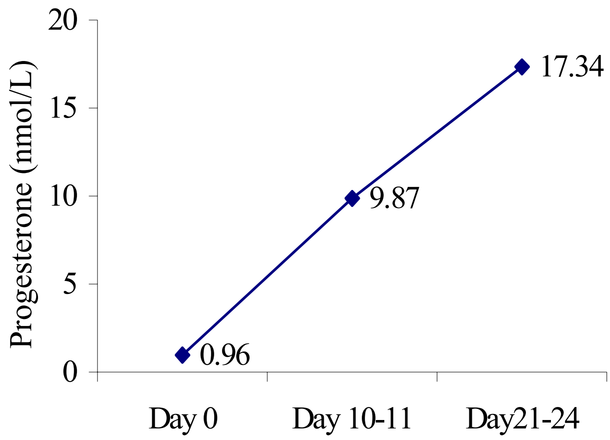

FIG. 1. Mean progesterone concentration at days 0, 10–12 and 21–24 after AI in animals that conceived.

The interval from calving to conception was influenced by the system of feeding on different types of farms, being significantly longer (180.4 ± 79.3 days) in cows provided grazing only, compared with those provided grazing and supplementary feeding 134.6 ± 44.4 days, $p < 0.05$). The mean interval in crossbred (116 ± 32.4 days) and Zebu cattle (122.4 ± 40.6 days) was shorter than that in local taurines (169.5 ± 59.6 days), but this difference was not significant.

TABLE IV. REPRODUCTIVE PERFORMANCE OF COWS AND HEIFERS SUBJECTED TO ARTIFICIAL INSEMINATION AFTER A NATURAL OR SYNCHRONIZED OESTRUS

AI done after:	Services (n)	Conceptions (n)	Mean interval to conception (d)	Overall conception rate (%)	Services per conception
Natural oestrus	56	29	125.4 ± 38.8	51.8	1.9
Synchronized oestrus	221	58	150.9 ± 62.8	26.2	3.8

The parity of cows and the month of calving did not significantly influence the interval to conception, but calvings from December to February were followed by shorter intervals than calvings at other times.

The mean conception rate to first service was 31.1% (n=83, including animals with no results from progesterone measurement) and the overall conception rate (OCR) was 31.4% (n=87). These two rates were similar because many cows that did not conceive to the first service could not be followed until subsequent conception. The OCR was significantly higher ($p < 0.005$) when AI was done after a natural oestrus (51.8%) than when it was done after a synchronized oestrus (26.2%). The overall mean number of services per conception was 3.2, with 1.9 after natural oestrus and 3.8 after synchronization. Large variations in OCR were observed between farms, ranging from 0 to 77%.

The OCR was significantly greater ($p < 0.01$) in crossbred cows (52%) than in Zebus (31.2%) or local taurines (27.97%). Semen from Girolando bulls had the highest OCR (43.3%), followed by semen from Holstein and Montbéliard (34.4%), Azawak (29.7%) and Gir (17.8%).

The level of education of AI technicians had a significant effect ($p < 0.03$) on OCR (ranging from 0 to 76.5%) but the length of experience had no effect. Self employed technicians achieved a higher OCR (39.2%) in comparison to government technicians (29.6%), but this difference was not significant.

Farms with dairy production as the sole or primary purpose achieved higher OCR ($p < 0.05$) than those with dual or triple objectives (Table V).

TABLE V. OVERALL CONCEPTION RATE ON FARMS WITH DIFFERENT OBJECTIVES IN CATTLE PRODUCTION

Objective	N° services	N° conceptions	Conception rate (%)	Services/conception
Dairy	25	15	60	1.7
Dairy/beef	102	31	30.4	3.3
Dairy/beef/draught	150	41	27.3	3.7

Parity, month of calving and month of AI did not have a significant effect on OCR but AI done during December to February appeared to result in higher OCRs (Fig. 2.).

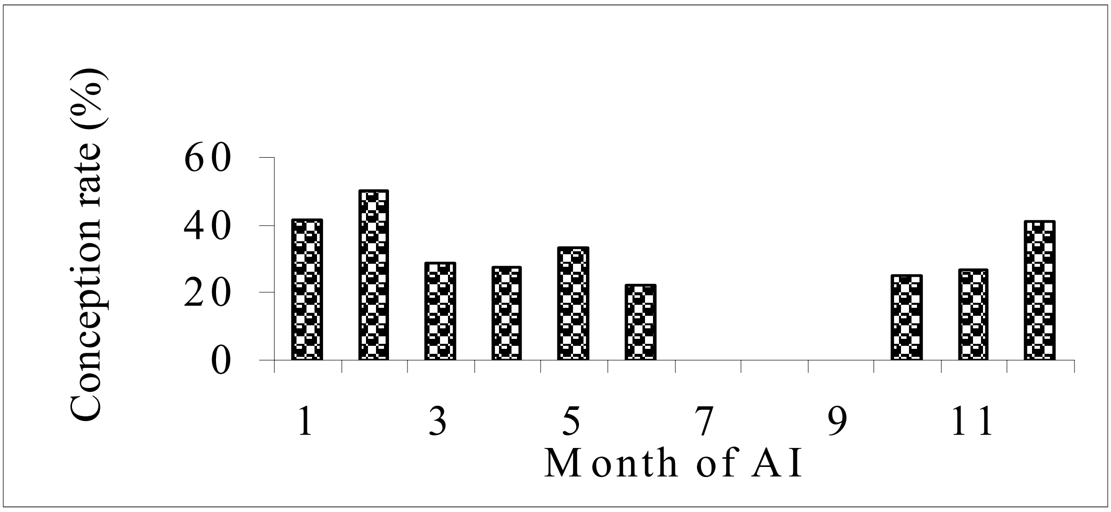

FIG. 2. Influence of the month when AI was done on overall conception rate.

3.3. Benefit-cost analysis (BCA)

The cost of measuring progesterone in one sample of milk was calculated by considering parameters such as variable direct costs, personnel costs and fixed costs. This was estimated to be €3.14 for the 'Self-coating' method, but could be reduced if the progesterone standards were prepared locally.

The costs (revenue foregone) due to a cow being open (non-pregnant) for one oestrous cycle (taken as 24 days) longer than the optimum open period was estimated by considering the loss occurring due to the cow not producing milk for one unproductive cycle plus the loss due to the proportional value of a calf for that period. The results for extensive and intensive systems of management are given in Table VI.

TABLE VI. ESTIMATED REVENUE FOREGONE DUE TO A COW BEING NON-PREGNANT AND UNPRODUCTIVE FOR ONE OESTROUS CYCLE

	Milk production (L)	Price (€/L)	Calf cost (€)	Total revenue foregone(€)
Extensive system	3	0.42	17.12	(3*0.42*24) + 17.12 = 47.36
Intensive system	8	0.42	20	(8*0.42*24) + 20 = 100.64

The impact assessment that was done through discussions and interviews with end-users of the N-PD technique indicated that farmers became aware that there was a method for early detection of non-pregnant cows and were interested in using it. They would adopt the method as a management tool and were agreeable to bearing the costs, provided that the results were returned to them in a timely manner. The AI technicians stated that the technology helped them to determine the causes of low conception rates, such as poor heat detection by the farmers and cows remaining acyclic after synchronization, and to take remedial measures to improve the success rate to AI.

4. DISCUSSION

This study, conducted in 14 farms belonging to three different farming systems, showed that the reproductive performance of cattle is below the expected level. The mean interval from calving to conception was 145.6 days, which is long when compared with those reported in developed countries. Furthermore, this total is a favourable estimate, because it did not include cattle that never conceived or took so long to conceive that they were not followed to the end of the study. Under these conditions, the interval between calvings will average around 14 months, whereas the aim of dairy farming is to have one calf per year in order to maximize the productive life of each cow. A large variation was found in relation of many factors like farm management, with shorter intervals ranging from 50 to 97 days in farms with intensive systems where supplementary feeding was practiced. Previous studies [5] have found a high correlation between the nutritional status of cows post partum and the length of the anoestrous period. For example, days to conception were significantly longer and conception rates were significantly lower in the dry season compared to the wet season, attributed partly to the poor nutritive value of pasture. In the present study, calvings from December to February were followed by shorter intervals to conception, confirming the effect of seasonal changes in quality and availability of pasture. Although concentrates were offered by some farmers, they were not fed in adequate quantities, resulting in negative energy balance and, thereby, affecting the calving to conception interval and conception rate. Farmers must therefore be sensitized to the need to reduce the interval from calving to conception by adequate feed supplementation.

The results from measurement of progesterone in the three samples collected after AI showed that only 26% of the animals conceived to a first service, while 42.6% were anoestrous or acyclic at the time of AI. The OCR was 31.4% and was significantly greater ($p < 0.005$) when AI was done after a natural oestrus (51.8%) than after a synchronized oestrus (26.2%). This indicates that a high proportion of animals that are subjected to oestrus synchronization are acyclic and do not ovulate after the treatment. The anoestrous state of the cattle also likely to be related to their nutritional status and needs to be addressed through extension programmes to educate farmers on proper feeding of animals before synchronization is done.

The OCR for synchronized cows recorded in the present study is very low when compared with previous reports of 48% for Baoulé cattle in Burkina Faso [6]. In order to improve the OCR to the level obtained in cows inseminated after natural oestrus one must ensure proper selection of animals based on their body condition score and cyclic status [7–9]. Other factors necessary for success include adequate training of inseminators and adoption of a synchronization regime that is suitable for the local conditions.

The cost of assaying one milk sample for progesterone was estimated to be €3.14. Attempts were made to determine the benefits of the early N-PD technique in terms of the advantages in shortening the open period. However, most farms did not have a system for recording production and economic data and several assumptions had to be made in making the calculations. It was estimated that the revenue forgone from an unproductive cow for 24 open days (considering mean milk production per day and calf loss) was €47.36 in extensive farms and €100.64 in intensive farms. In comparison to the RIA technique, which can detect non-pregnant cows at 21–24 days, rectal palpation can detect pregnant and non-pregnant cows but this can be done only at 60 to 90 days after AI. In the case of cows remaining non-pregnant for this extended period, the total revenue foregone will be more than €160.76 for extensive systems and €403.04 for intensive systems.

The project achieved its objectives in terms of determining the efficiency of AI services and identifying reproductive problems on different types of farms. It helped to identify action points to improve cattle reproduction in order to increase the profitability of farmers and reduce poverty.

Impact begins to occur when there is a behavioural change among target beneficiaries. The farmers participating in this project indicated their interest to continue participation and even bear the costs of the progesterone assay. However, an important prerequisite was that they should receive timely feedback on the results in order to take appropriate decisions. It is concluded that the RIA technique for early N-PD can be integrated in to AI programmes in order to increase their effectiveness, reduce the unproductive period of dairy cows and increase the economic benefits to farmers.

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IMPROVING ARTIFICIAL INSEMINATION SERVICES FOR DAIRY CATTLE IN ETHIOPIA

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Abstract

Studies to determine the current status and efficiency of artificial insemination (AI) were undertaken by the National Artificial Insemination Centre (NAIC) of Ethiopia on 52 dairy farms (4 large and 48 small-to-medium farms) located around Addis Ababa. Milk samples were collected from 417 cows on the day of AI (day 0), and on days 10–12 and 21–23 after AI. A total of 1085 samples were assayed for the concentration of progesterone using radioimmunoassay (RIA). Data pertaining to the farm, inseminated cow, the inseminator and semen batch were recorded. Rectal palpation was done to check for pregnancy two months after AI. The overall mean interval from calving to first service was 161.7 ± 139.8 days. Cows that calved during March to August, coinciding with wet weather when the availability and quality of feed is good, had shorter intervals to first service than those that calved during the rest of the year. Results from RIA showed that 89% of the cows had low progesterone on day 0, indicating that they were in the follicular phase or anoestrous. However, only 49% of the cows had elevated progesterone on day 10, indicating that an ovulatory oestrus had occurred at the time of AI. The results from all three milk samples indicated that 45% of the cows were likely to have conceived, but only 39% were later confirmed pregnant by manual palpation. A survey was done on seven medium to large farms on the costs and benefits of a service for early non-pregnancy diagnosis and infertility management using progesterone RIA. The overall mean calving interval was 435 days, which was 70 days longer than the optimum interval of 365 days. In most farms, 50% or more of the total expenses were for feed purchases, with expenses for health care and AI services accounting for only 5%. The profit, as a percentage of income, ranged from -4% to 50% in the seven farms. The cost of determining the progesterone concentration in one milk sample was calculated to be $8, of which 43% was accounted for by variable direct costs for RIA consumables. The average loss of milk due to extra days open was 827 litres per cow per lactation, equivalent to $207. Thus, the use of progesterone RIA to reduce the calving interval and overcome this loss would be highly cost-effective.

1. INTRODUCTION

The technology of artificial insemination (AI) for cattle was introduced at the farm level in Ethiopia over 35 years ago as a tool for genetic improvement. The efficiency of the AI program in the country, however, has remained at a very low level. Apart from the infrastructure, managerial and financial constraints, problems associated with reproductive management of cattle, such as poor heat detection, improper timing of inseminations and embryonic deaths are the major factors that limit the efficiency. Improving the system used for monitoring and follow-up of the outcome of AI and increasing the efficiency of AI services could considerably improve the productivity of dairy cattle.

Reproductive efficiency in dairy cattle is directly affected by the calving interval, which is composed of the time between calving and conception (the 'open' period) and the duration of pregnancy [1]. In order to achieve a short calving interval the cow must resume cyclicity at an early stage during the postpartum period and the farmer must detect heat accurately and have the cow inseminated at the correct time [2]. Cows that do not conceive and are not identified at the next heat period will have long open periods, leading to long calving intervals. Thus early identification of non-pregnant cows is essential to minimize the number of days that they remain open.

The concentration of progesterone in milk or blood is closely related to the estrus cycle of cattle [3]. The accurate determination of progesterone levels using the radioimmunoassay (RIA) technique can be applied to monitor postpartum ovarian activity and confirm estrus, and to diagnose non-pregnancy, embryonic death and ovarian disorders [4].

The sustainable application of such modern technology in the dairy development program would be possible only where cost recovery mechanisms are practiced. In order to sustainably deliver or sell the service in any development enterprise the true expenses of different activities have to be known and the prices of items or services sold must be covered from the incomes. But this has not been the practice in Ethiopia up to now, where services have been provided free of charge or with high subsidies from government funds.

From the beginning of the year 2001, Ethiopia has been participating in a regional Technical Cooperation project supported by the International Atomic Energy Agency (IAEA) on "Increasing and improving milk and meat production'', which has the objective of using progesterone measurement by RIA to (a) monitor and improve the efficiency of AI, and (b) provide an early non-pregnancy diagnosis (N-PD) service to farmers. This objective is in line with that of the National Livestock Development Programme of the country, which also aims at achieving increased production in milk and meat through the provision of cost effective and efficient AI and animal health services. In the past, many projects have made substantial progress in improving the efficiency of AI service, but the improvements have not been sustainable after the extra finances available through such projects ceased. Hence activities under the IAEA project included the introduction of a system for cost recovery, in order to sustain the continuation of services to farmers.

This paper presents results on the establishment of the RIA technique at the National Artificial Insemination Centre (NAIC), use of progesterone measurement in milk to study the reproductive performance of cattle bred by AI, an assessment of the impact of project activities and the findings of a survey performed to determine the feasibility of a cost recovery system for the sustainable use of the technology.

2. MATERIALS AND METHODS

2.1. Milk sampling and data collection

In order to ensure regular collection of milk samples from the field, project activities were concentrated in and around the Addis Ababa area. The study area is situated at latitude 9°3' North and longitude 38°43' East, and an altitude of 2408 meters above sea level. The average annual temperature is 16°C. The major rainy season extends from the end of May to late September, while the minor rainy season extends from the end of March to the end of May. The mean annual rainfall is 1201 mm. According to a census undertaken by the Addis Ababa Regional Agriculture Bureau in 1996, the area has a total of 5167 small, medium and large dairy farms, which produce 34 649 450 litres of milk annually.

The study was conducted on 52 dairy farms, comprising two large government farms (Holetta and Adaberga), two large private farms (Sebeta and Genesis) and 48 medium and small private farms (Table I). Most of the animals monitored during the study were Holstein-Friesian or highly upgraded local breeds. All animals in the large farms were kept indoors and stall-fed during the night and allowed to graze and exercise during daytime. Animals in small farms were kept indoors at al times and stall-fed only. Feed ingredients that were commonly used in all the farms included native hay, cereal straws, green grass (during the wet season), mill by-products, and oil seed cakes. The type and composition of ingredients allocated to cows varied considerably between the farms.

TABLE I. NUMBERS OF AI TECHNICIANS (AIT) AND COWS INCLUDED IN THE STUDY FROM THE DIFFERENT TYPES OF FARMS AND THE NUMBERS OF MILK SAMPLES COLLECTED

Farms	No. of AIT	No of cows	No. of milk samples			
			Day 0 (day of AI)	Days 11–13 after AI	Days 21–23 after AI	Total
Holetta	2	375	375	325	329	1029
Adaberga	1	70	70	40	30	140
Sebeta	2	60	60	56	44	160
Genesis	1	14	14	7	7	28
Medium & small (48)	6	170	170	134	118	422
Total	12	689	689	562	528	1779

Three milk samples were collected from each inseminated cow. The first sample was taken on the day of AI (day 0), the second between 10 and 12 days after AI, and the third sample between 21 and 23 days after AI. The AI technicians were provided with bottles containing a tablet of Sodium Azide as preservative for collection of milk samples and a recording format for collecting the relevant data. AI Technicians working close to the NAIC submitted the data and the milk sample directly to the RIA laboratory at the NAIC. Others who worked far away from the centre kept the sample at room temperature for a maximum of one week, after which it was collected and brought to the RIA laboratory in an icebox. A total of 1779 milk samples were collected from 689 cows for progesterone measurement in order to assess their reproductive status.

On arrival at the laboratory the milk samples were registered and centrifuged at 2000 RPM for 15 minutes to separate the fat from the skim milk. The tubes were kept at 4°C for 15 minutes to harden the fat layer, the skim milk was aspirated and stored at -2°C until assayed. Progesterone was measured using the 'self-coating' RIA technique developed by the Joint FAO/IAEA Programme [5]. Initially, all necessary equipment and chemicals including the radio-iodinated progesterone tracer were provided by the IAEA. Subsequently, the RIA laboratory at NAIC produced its own milk standards and quality control samples for the assay. A single-well gamma counter was used to determine the radioactivity in the tubes. Progesterone concentrations >3 nmol/L were considered as 'high', indicating the presence of luteal phase ovarian activity, while those <1 nmol/L were considered as 'low', indicating absence of luteal activity (follicular phase or anoestrus). Intermediate values (1–3 nmol/L) were considered inconclusive.

Information collected regarding the farm, cow, heat period, insemination technique, semen batch and inseminator were entered into the AIDA Africa application [6], a computer software package designed to store data and compile reports related to AI.

2.2. Cost-benefit analysis

A survey was conducted on 7 dairy farms that were representative of the different farm sizes (ranging from 2 to 120 breedable cows) present within a radius of 70 km from the RIA laboratory at NAIC. They were selected on the basis of having improved exotic breeds, adopting good management standards and interested in the application of progesterone RIA for improving the reproductive management. The objective was to determine the technical feasibility and economic value of the programme.

Data was collected through visits to the farm to access farm data (technical and economical) and interviewing the farmer, herdsmen and AI technicians. Different cost areas associated with the RIA analysis were calculated using the format developed during a previous Task Force Meeting of the AFRA project held in Senegal. Costs for feed, labour, health care, supplies and maintenance for each farm were calculated and analysed to determine the expenses. The reproductive efficiency of a cow was assessed from the number of days open (calving to conception interval) and productive efficiency was determined from the lactation yield. The potential benefits from reduced days open and increased lactation yield were compared with the increased costs due to the early N-PD service using RIA.

2.3. Cost-recovery strategy

A project under the National Livestock Development Programme in Ethiopia had conducted a study in 2001 to determine the potential for cost recovery in the provision of services to farmers for AI, animal health and forage seed supply [7]. Since the early N-PD service using RIA could also be integrated in to the national AI service program, a cost recovery strategy for this technology was developed using a similar approach.

2.4. Training and education

During the project period various training workshops and seminars were held for farmers, AI technicians and livestock professionals involved in the project activities. Farms under investigation were visited on several occasions and discussions were conducted on the management and reproduction of dairy cattle. Towards the end of the project period a one-day seminar was organized to discuss the outcome of the project activities and the future sustainability of the programme, with the participation of farmers, AI Technicians and livestock professionals from the Ministry and the AI centre.

3. RESULTS

3.1. Reproductive parameters

Records were analysed for a total of 417 cows from the four large farms and 48 small to medium farms used in the study. The mean intervals from calving to first service are shown in Table II. The two government farms (Holetta and Adaberga) had the shortest intervals. The range of intervals for the different farms was quite wide, indicating that there is wide variability in the post-partum anoestrous period in the cattle sampled.

TABLE II. INTERVALS FROM CALVING TO FIRST SERVICE IN THE DIFFERENT FARMS

Farms	No. of cows	Mean interval (days)	S.D
Holetta	238	142.9	118.8
Adaberga	34	161.6	121.1
Sebeta	17	182.2	124.1
Genesis	12	265.7	255.7
Medium & small (48)	116	192.6	129.2
Overall	417	161.7	139.8

The interval from calving to first service was influenced by the month of calving (Table III). Cows that calved during the period March to August had shorter intervals than those that calved during the rest of the year. This period coincides with the season of wet weather, when the availability and quality of feed is better in highland regions of Ethiopia.

TABLE III. INTERVALS FROM CALVING TO FIRST INSEMINATION BY MONTH OF CALVING

Month of calving	No of cows	Mean interval (days)	S.D
January	57	186.8	127.3
February	46	175.6	189.1
March	47	107.6	58.7
April	45	150.3	110.1
May	32	125.5	74.3
June	31	159.0	177.5
July	25	108.7	162.1
August	24	88.9	70.9
September	17	203.3	126.1
October	34	225.3	50.8
November	20	295.1	329.8
December	39	165.5	49.8
Overall	417	161.7	139.8

The number of inseminations carried out during the different months according to the service number is given in Table IV. The months from April to October recorded higher numbers of inseminations than other months. Of the total number of cows inseminated, 47.5% were submitted for repeat inseminations.

TABLE IV. NUMBER OF INSEMINATIONS DONE ACCORDING TO THE MONTH AND SERVICE NUMBER

Service Number	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
1			6	45	20	28	20	25	39	26	6	4	219
2			3	11	8	12	15	13	13	8	2	1	86
3			3	7	10	3	6	6	5	8	3	3	54
4			3	6	5	6	2	8	6	3	2	2	43
5					3	2	1		1	1	1	1	10
6					1	1		2		1			5
Total			15	69	47	52	44	54	64	47	14	11	417

TABLE V. NUMBERS OF MILK SAMPLES WITH LOW (<1 NMOL/L), INTERMEDIATE (1–3 NMOL/L) AND HIGH (>3 NMOL/L) PROGESTERONE CONCENTRATION ON DAYS 0, 10–12 AND 21–23 AFTER AI.

Day of sampling	No. of samples	Progesterone concentration		
		Low	Intermediate	High
Day 0	417	370 (89%)	22 (5%)	25 (6%)
Day 11–13	346	79 (23%)	97 (28%)	170 (49%)
Day 21–23	322	118 (37%)	59 (18%)	145 (45%)
Total	1085	567	178	340

From the total of 1779 milk samples collected during the project period, 1085 samples from 417 cows were assayed for progesterone. The percentages of samples with progesterone values that were classified as low (<1 nmol/L), intermediate (1–3 nmol/L) and high (> 3 nmol/L) at the three times of sampling are given in Table V. On the day of AI (day 0), 89% of the cows had low progesterone, indicating that they were not in the luteal phase, and therefore probably in oestrus or anoestrus. At the sampling on day 21–23, 145 cows (45%) had high progesterone, indicating that they may have conceived. However, only 125 cows (39%) were later confirmed pregnant by manual palpation done two months after AI. Further interpretations done on the results from progesterone measurement on one, two, or three samples and in combination with manual pregnancy diagnosis are presented in Tables VI, VII and VIII.

TABLE VI. DIAGNOSIS BASED ON PROGESTERONE CONCENTRATION IN ONE SAMPLE OF MILK COLLECTED AT THE TIME OF ARTIFICIAL INSEMINATION (AI)

Progesterone	No. of cows (%)	Interpretation
Low	370 (89)	AI done at a time other than the luteal phase (i.e. probably during oestrus or anoestrus)
High	25 (6)	AI done during the luteal phase or pregnancy
Intermediate	22 (5)	AI done too early or too late relative to oestrus; other clinical data is required for interpretation
Total	417	

TABLE VII. DIAGNOSIS BASED ON PROGESTERONE CONCENTRATION IN TWO SAMPLES OF MILK COLLECTED ON THE DAY OF AI AND 11–13 DAYS AFTER AI

Progesterone		No. of cows (%)	Interpretation
Day 0	Day 11–13		
Low	High	170 (49)	AI done at an ovulatory oestrus
Low	Low	68 (20)	Anoestrus, anovulation or short luteal phase
High	High	4 (1)	AI on pregnant animal or luteal cyst
High	Low	7 (2)	AI done during luteal phase
*	*	97 (28)	* At least one of the samples showed an intermediate value; other clinical data is required for interpretation
Total		346	

TABLE VIII. DIAGNOSIS BASED ON PROGESTERONE CONCENTRATION IN THREE SAMPLES OF MILK COLLECTED ON THE DAY OF ARTIFICIAL INSEMINATION, 11–13 AND 21–23 DAYS LATER

Progesterone			Pregnancy diagnosis	Frequency (%)	Interpretation
Day 0	Day 10–12	Day 22–23			
Low	High	High	Positive	125 (39%)	Pregnant
Low	High	Low	Negative	118 (37%)	Non-fertilization, early embryonic mortality or post AI anoestrus
Low	High	High	Negative	16 (5%)	Late embryonic mortality (>day 16), luteal cyst or persistent CL
High	High	High	Positive	4 (1%)	AI on pregnant animal or luteal cyst
*	*	*	Positive/ Negative	59 (18%)	* At least one of the samples showed an intermediate value; other clinical data is required for interpretation
Total				322	

3.2. Cost-benefit analysis

The herd size, reproductive performance of cows, the average lactation yield per cow and the value of total milk produced per farm for the seven farms used in this component of the study are given in Table IX. The average calving interval was 435 days. Assuming that the optimum calving interval is 365 days, this calving interval was longer than that by 70 days. The total income from milk was compared with the total expenses for the major components on each farm (Table X). In most farms, 50% or more of the total expenses were for feed purchases. Expenses for health care and AI services accounted for only 5% of the total cost. The profit, as a percentage of income, ranged from -4% to 50% in the seven farms.

The cost of determining the progesterone concentration in one milk sample was calculated to be about $8 (Table XI). Of this, 43% was accounted for by variable direct costs (consumables for the RIA laboratory). The variable indirect costs included expenses for sample collection, storage and providing feed-back information. The fixed costs included RIA laboratory equipment including the gamma counter.

The average loss of milk due to extra days open was 827 litres per cow per lactation, corresponding to an economic loss of $207. The total loss of income from the seven farms due to these extra open days was equivalent to $162 814 per lactation (Table XII).

The cost of providing a service to farmers using progesterone RIA was calculated for early N-PD and infertility investigations based on one or two samples, on the presumption that all cows on each farm would be tested. The potential gain in income from such a service, assuming that it would achieve a mean calving interval of 365 days on each farm, is given in Table XII.

TABLE IX. HERD SIZE, REPRODUCTIVE PERFORMANCE AND MILK PRODUCTION IN THE SEVEN FARMS SURVEYED

Farm	Herd size	No. of cows	Calving to AI (d)	Calving to conception (d)	Calving interval (d)	Extra days open	Avg. lactation yield (L/cow)	Total farm yield (L/year)	Value of milk[a] (Birr/year)
Holetta	459	233	120	136	450	85	3638	847 654	1 695 308
Adaberga	302	162	89	115	436	71	2600	421 200	842 400
Mohamed	360	150	98	142	428	63	3662	549 300	1 098 600
Stella	308	132	135	152	445	80	3500	462 000	92 400
Genesis	107	47	106	130	427	62	4779	225 553	451 106
Eshete	238	95	142	175	450	85	4556	432 820	865 640
Aregash	13	6	120	145	410	45	4680	28 080	56 160
Total	1787	825						2 966 607	5 933 214
Average	255	118	116	142	435	70	3919	423 801	847 602

[a]Value of milk = Birr 2.00/L; $1 = Birr 8.00

TABLE X. MAIN COMPONENTS OF EXPENSES AND THE INCOME FROM MILK IN THE SEVEN FARMS

Farm	Farm Expenses (Birr)[a]					Total Expenses (Birr)	Income from Milk (Birr)	Profit	
	Feed	Health and AI	Labour	Supplies and maintenance	Others			Birr	% of income
Holetta	492 718 (42%)	60 936 (5%)	364 317 (31%)	220 625 (19%)	30 000 (3%)	1 168 596	1 695 308	526 712	45
Adaberga	288 474 (43%)	49 210 (7%)	132 559 (20%)	185 569 (27%)	20 000 (3%)	675 812	842 400	166 588	25
Mohamed	486 780 (61%)	23 940 (4%)	153 711 (16%)	95 760 (12%)	20 000 (3%)	798 000	1 098 600	300 600	37
Stella	685 192 (71%)	38 602 (4%)	153 711 (16%)	67 554 (7%)	20 000 (2%)	965 059	924 000	-41 059	-4
Genesis	204 887 (67%)	15 840 (5%)	36 317 (12%)	35 000 (11%)	15 000 (5%)	307 044	451 106	144 062	47
Eshete	413 200 (54%)	4500 (6%)	200 000 (26%)	81 200 (11%)	20 000 (3%)	759 400	865 640	106 240	14
Aregash	24 466 (66%)	2000 (5%)	3840 (10%)	5600 (15%)	1 500 (4%)	37 406	56 160	18 754	50
Total	2 595 717	235 528	1 062 264	691 308	126 800	4 711 317	5 933 214	1 221 897	
Average	370 816 (55%)	33 647 (5%)	151 753 (22%)	98 758 (15%)	18 071 (3%)	673 045	847 602	174 557	26

[a]$1 = Birr 8.00

TABLE XI. COST ESTIMATE FOR MEASURING PROGESTERONE IN ONE SAMPLE OF MILK BY RIA

Cost area	Birr	$	% of total cost
Variable direct cost (consumables)	28.56	3.36	43%
Variable indirect cost (others)	20.48	1.45	18%
Personnel	12.32	2.45	31%
Fixed costs	5.61	0.66	8%
Total	67.00	8.00	100%

[a]$1 = Birr 8.00

TABLE XII. COST-BENEFIT ANALYSIS ON THE USE OF RIA FOR MEASURING PROGESTERONE

Farm	Average milk/cow (L/d)	Extra open days[a]	Milk yield lost		Income lost		Cost for RIA ($)		Income gained[c] ($)			
			Per cow (L)	Total farm (L)	Birr	$[b]	One sample	Two samples	One sample	%	Two samples	%
Holetta	12	85	1020	237 660	475 320	55 920	1864	3 728	54 056	97	52 192	93
Adaberga	8	71	568	92 016	184 032	21 651	1296	2 592	20 355	94	19 059	88
Mohamed	11	63	693	103 950	207 950	24 459	1200	2 400	23 259	95	22 059	90
Stella	10	80	800	105 600	211 200	24 847	1056	2 112	23 791	96	22 735	91
Genesis	10	62	930	43 710	87 420	10 285	376	752	9 909	96	9 533	93
Eshete	13	85	1105	104 975	209 950	24 700	760	1 520	23 940	97	23 180	94
Aregash	15	45	675	4 050	8 100	953	48	96	905	95	857	90
Total		491	5791	691 961	1 383 922	162 814	6600	13 200	156 215		149 615	
Average		70	827	98 852	197 703	23 259	943	1 886	22 316	96	21 374	92

[a]Assuming an optimum calving interval of 365 days; [b]$1 = Birr 8.00; [c]Assuming that the RIA service will eliminate extra open days

3.3. Cost-recovery strategy

The cost-recovery strategy shown in Table XIII was developed in accordance with the approach that was adopted under the National Livestock Development Programme of the country, based on a study conducted to consider the potential for cost recovery in the area of AI, animal health and forage seed supply. It was considered that the RIA service could also be integrated in the national AI service program. The basic principle of the strategy is that when the number of services or the sample size increases the price of the service decreases, because the fixed expenses per unit become smaller. The strategy was based on the assumption that the number of farms using in the service would be limited during the initial stages, but would increase progressively as its advantages became recognized by the farmers.

TABLE XIII. TOTAL EXPENSES PER NUMBER OF SAMPLE PER YEAR AND EXPECTED LEVEL OF COST RECOVERY

Year	No. of Samples	Fixed cost (Birr)	Variable cost (Birr)	Total cost (Birr)	Cost per sample		Cost recovery level		
					Birr	$[a]	Birr	$	%
1	200	5200	8200	13 400	67	7.88	10	1.2	10%
2	300	5200	12 300	17 500	58	6.82	13	1.5	20%
3	400	5200	16 400	21 600	54	6.35	20	2.4	30%
4	500	5200	20 500	25 700	51	6.00	30	3.5	45%
5	600	5200	24 600	29 800	50	5.88	40	4.7	60%
6	700	5200	28 700	33 900	48	5.64	55	6.5	80%
7	800	5200	32 800	38 000	47	5.52	67	8.0	100%

[a]Birr 8.00 = $1.00

3.4. Training and education

The training workshops and seminars that were held for farmers, AI technicians and livestock professionals involved in the project activities are shown in Table XIV. In addition, on-farm training and provision of information was done for farmers and AI technicians during farm visits in connection with project activities.

TABLE XIV. TRAINING ACTIVITIES CONDUCTED FOR FARMERS, AI TECHNICIANS AND LIVESTOCK PROFESSIONALS

Activity	Number of participants	Duration of each (days)
Sensitization of AI technicians	12	3
Training of farmers	52	1
Seminar for AI professionals	8	1
Seminar for livestock professionals	15	1
Total	159	6

4. DISCUSSION

4.1. Establishment of RIA technology and interpretation of data

A laboratory for measuring progesterone using the RIA technique was successfully established at the NAIC. The field activities for collecting milk samples and recording relevant data relating to AI were conducted on four large farms and 48 small to medium sized farms. The AI technicians who provide services to these farms were given the necessary training and materials to undertake this work as part of their routine duties. Although it had been planned that three samples of milk would be collected from each inseminated cow, this was not always possible. Thus, 417 milk samples were collected from cows on the day of AI (day 0), but of these, only 346 and 322 cows had samples collected on days 10–12 and 22–24, respectively. The reasons for missing some samples include inadequate motivation or inability of the AI technicians to return to these farms on the assigned dates, improper labelling or handling of samples, shortage of transport facilities to bring the sample to the RIA laboratory and lack of proper animal identification and records to ensure follow-up of inseminated cows on some smallholder farms. Therefore, the AI technicians and farmers need to be continuously encouraged to submit the required milk sample and necessary farm information to the RIA laboratory for timely analysis. Timely provision of feed-back information to farmers on the results and the follow-up actions to be taken are also essential in order to maintain a continual stream of sample submissions.

Out of the 417 cows sampled on day 0, 370 (89%) had basal (<1 nmol/L) progesterone concentration in milk, indicating that AI was probably done at the correct time, at least when the cows were without an active corpus luteum. This result shows that the heat detection by the farmers, the insemination and milk sample collection by the AI technicians were satisfactory. However, the remaining 11% of animals had intermediate or high levels of progesterone, indicating a need to further sensitize both the AI technicians and the farmers regarding the proper observation of the heat signs, time of insemination and proper record keeping.

Normally, calving is followed a period of acyclicity of 4–12 weeks. However, the mean interval from calving to first service in the farms studied was 161 days. This result indicates that most of the cows had relatively long postpartum anoestrous periods, thereby prolonging the calving interval. Further, the conception rate based on RIA results was 45%, while confirmed pregnancy rate based on rectal palpation was 39%. These results show that the reproductive performance of cows on the farms studied is low when compared with previous reports from Ethiopia and elsewhere [8–11]. This deficiency could be due to many factors, including poor management, feeding and heat detection, incorrect time of insemination and stress due to unfavourable season. The observed seasonal fluctuations in reproductive efficiency in the present study indicates an obvious advantage for planned seasonal breeding so as to avoid a decline in reproductive performance associated with unfavourable seasons. Further factors which result in delay from calving to conception include the efficiency of the inseminator, quality of the semen used and postpartum diseases.

4.2. Potential for the application of RIA technology and cost-recovery

In any dairy farming system, the most economically favourable results are achieved when the cows have a calving interval of 12 months [1, 12]. In these herds, the average calving interval was 435 days, which exceeded the optimal interval by 70 days. When we compared the total costs to the income obtained from the milk sales, the profit balance was positive in 6 farms and ranged from 14–50%. One farm (Stella) had a negative profit balance of 4%. This farm spent 71% of its costs on feed, but the expected increase in productivity was not achieved. This could be due to poor genetic potential of the cows, combined with poor resource allocation and management of the farm.

In the cost benefit analysis assumed that the progesterone assay service could be utilized for any of the following purposes, with the objective of reducing the long calving intervals:

- Monitoring cyclicity: by checking the milk progesterone level during the postpartum period at 10 days intervals for 4 to 12 weeks, to detect elevated levels that signify the onset of cyclicity;

- Verifying the timing of A1: using one sample taken on the day of AI to check for the presence or absence of luteal activity;

- Early N-PD: using one sample taken 21–23 days after AI;

- Monitoring the result of treatment for follicular cysts: using one sample taken 10 days after treatment to check for luteal activity, indicating successful treatment.

Farms using these reproductive control measures through the application of progesterone RIA are expected to achieve the optimum calving interval of 365 days. This assumption was the basis for the cost-benefit analysis done in the present study. The income forgone when a cow was open for more than 85 days after calving was mainly due to less milk produced per day of that lactation and the longer interval necessary to produce a calf. According to our results, the average loss of milk due to extra days open was 827 litres per cow per lactation, equivalent to $207. The total loss of income from the seven farms due to these extra open days was equivalent to $162 814. If this loss were to be corrected by the use of progesterone RIA, the overall gain in income would be around 90% of this value. As clearly shown in Table XII, using either one sample to perform early N-PD at 21–23 days after AI or two samples for diagnosing fertility problems would be very cost effective for the farmers.

With regard to cost recovery for the provision of progesterone RIA services, it was considered that a scheme to introduce this gradually would be necessary. It should commence with government farms and a few selected progressive private farms. Since the NAIC is mandated to implement such a development activity, it is recommended that the cost recovery exercise should start at the Holetta bull dam farm, which is under the management of the NAIC. In our opinion, a charge of Birr 10 ($1.20) per sample at the initial stage would be realistic. The projected time period for full cost recovery is 7 years, with the charge per sample increasing 10 to 20% per year. This strategy is based on the assumption that the demand for the RIA service will increase progressively each year.

On the basis of our results presented above, it can be concluded that the cows involved in this study have shown low reproductive efficiency, characterized by long intervals from calving to first AI (161.7 days) and low conception rate (45%). This result could be attributed to poor management practices including inadequate and unbalanced nutrient supply, poor heat detection, incorrect timing of insemination, inappropriate housing and postpartum diseases.

The present study has demonstrated the potential value of a progesterone RIA service for dairy farmers, in combination with recording, analysis and reporting using the AIDA software program. It offers an easy and precise way for assessing the ovarian activity and characterizing sub-fertility in dairy cows so that prompt measures could be taken before incurring much cost in terms of reproductive management, feed and labour.

In addition to such measures, efforts should be made to improve the overall standards of husbandry and management of dairy farms, particularly in the urban and peri-urban sectors. Further studies are also warranted to examine the effects of environmental and management factors on reproductive performance of dairy cows in such relatively intensive production systems.

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THE USE OF RADIOIMMUNOASSAY IN EARLY DIAGNOSIS OF NON-PREGNANCY IN SMALLHOLDER DAIRY HERDS OF KENYA

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Abstract

Kenya's smallholder dairy sector produces 85% of the total milk marketed in the country. The majority of cows in this sector have long calving intervals, leading to low lifetime production of milk and calves. Determining non-pregnant cows early by measuring progesterone levels at 23 days after artificial insemination (AI) can help reduce calving intervals, thus improving productivity. A study was conducted on 481 cows subjected to AI, to determine the timeliness of AI, cyclicity and pregnancy status. Milk samples (10 mL) were collected from each cow on day 0 (day of AI) and on days 13 and 23 after AI. Data pertaining to the farm, cow, heat period, AI technician and semen batch were recorded. Progesterone concentration was measured in skim milk using a solid-phase radioimmunoassay. Rectal palpation was done 90 days after AI to confirm the predictions made from hormone assay. The overall conception rate (CR) was 53%. Mean progesterone levels in pregnant cows were 0, 5.3 and 10.1 nmol/L at the three stages of sampling, while in non-pregnant cows they were 0.59, 2.69 and 0.46 nmol/L, respectively. Low progesterone was found in 83% of the cows at the time of AI, but of these 46% did not conceive, indicating that they were either non-cyclic, had fertilization failure or suffered early embryonic loss. At the time of AI 14% of the cows were in the luteal phase, while 32% were not cycling. Cows were inseminated on average 19 hours after onset of heat. The interval from calving to first AI averaged 5 months. There were no differences between breeds in CR, but cows inseminated during April to June (long rain period) had higher CR than those inseminated during July to September. The accuracy for prediction of non-pregnancy by progesterone assay was 99% and that for predicting pregnancy was 95%. It is concluded that this technique can identify open cows by day 23 after AI, thus reducing the time required to rebreed, which will lead to shorter calving intervals.

1. INTRODUCTION

The livestock sector in Kenya accounts for about 10% of the GDP and over 30% of the farm-gate value of agricultural commodities [1]. It employs over 50% of the agricultural labour force and provides substantial raw materials for the local dairy, meat, hides and skins industries. The sector annually produces a total of 2.4 billion liters of milk [1] of which 85% is produced by smallholder farmers with an average farm size of 2 ha practicing mixed farming in a diversified production system [2].

Dairy farming also contributes to the beef industry through slaughter of cull cows and steers. Kenya has a dairy cattle population of 3.4 million that occupies 30% of the land in high and medium potential areas. Out of the total milk produced from these cattle, 62% is marketed while 38% is consumed at home [3]. In addition, the cattle provide manure and draught power, and convert crop residues and by-products into useful animal protein. The demand for milk in Kenya is expected to continue increasing due to population increase, improved incomes and accelerated urbanization, and is expected to double by the year 2010 [3]. Although there has been some increased production from smallholder farms, this can be attributed to extended land use rather than improved productivity per cow per unit land area.

The average milk yield of cows on smallholder farms is 5.8 kg per day and the shape of the lactation curve does not show a peak as expected between days 40–70 after calving [4]. These findings indicate that poor nutrition, especially insufficient dry matter intake, is a major limiting factor [5]. The low milk production by cows is an important constraint to optimal calf growth, resulting in delayed age at first calving, which averages 41 months.

The second major constraint reported is the long calving interval, which averages 633 days with a range of 308–1256 days [4]. The calving interval is influenced by many factors, including the number of days from calving to conception, conception rate (CR) at first insemination and the number of inseminations per conception. Long calving intervals lead to extended lactation lengths, estimated at 16 months on average, instead of the usual 305 days. This causes yearly losses to farmers as a result of decreased annual milk yield, reduced number of calves over the lifetime of the cow and reduced genetic progress in the herd due to having fewer potential replacements available for selection.

Early identification of non-pregnant cows after insemination is of economic importance. If conception does not occur, the corpus luteum (CL) regresses and the concentration of progesterone in blood drops to basal levels, permitting follicular growth and maturation to proceed, resulting in the cow returning to oestrus. On the contrary, if conception occurs, the CL persists and progesterone levels remain elevated, preventing the cow from returning to oestrus [6]. Therefore, an animal not returning to oestrus after service is assumed to be pregnant, but confirmation requires manual palpation by a trained veterinarian or technician between days 60–90 after AI. However, sensitive immunological methods of measuring progesterone in biological fluids including milk and serum are now available and can be used for early diagnosis of non-pregnancy between days 21–23 after AI [7].

The objective of this study was to measure levels of progesterone in milk of dairy cattle to determine the success rate of AI, identify factors that result in low success rates and to recommend interventions that can improve conception rates and lead to reduced calving intervals.

2. MATERIALS AND METHODS

The study was conducted in Nakuru district of Kenya in 10 farming groups comprising three private large-scale farms, three private AI practitioners and four community based AI schemes drawing their membership from smallholder farms (Table I).

TABLE I. DESCRIPTION OF THE TEN FARMING GROUPS INCLUDED IN THE STUDY

Farming Group	Description
Delamere Estate	Private Farm
N'gera Farms	Private Farm
Rumwe Farmers Cooperative	Community AI
Bahati Farmers Cooperative	Community AI
Menegai Agrovet	Private AI
Damside Dairies	Private AI
Ngorika Dairies	Community AI
Itherero Self Help Group	Community AI
Subukia Farmers Society	Community AI
Individual farms	Private AI

Collaborating farmers and AI technicians were given printed forms to record data on individual cows subjected to AI, and 10 mL bottles containing a sodium azide tablet as preservative to collect milk samples. They were requested to collect three samples from each cow, the first on the day of AI (denoted as day 0), the second on day 12 after AI, and the third on day 22 after AI. The individual cow data sheets and samples were submitted to the laboratory progesterone assay. The breeds of cattle sampled were Friesian, Ayrshire, Guernsey and Jersey.

On arrival at the laboratory, the milk samples were centrifuged at 3000 RPM for 30 minutes to separate the fat from the skim milk and placed at 4°C to harden the fat. The skim milk was then removed using a Pasteur pipette and stored at 4°C until assayed for progesterone by the 'self-coating' radioimmunoassay (RIA) technique using the method developed by the Joint IAEA/FAO Programme [7]. The radioactive tracer was obtained from South Africa and the milk standards were prepared locally. A single-well gamma counter was used to determine the radioactivity in the tubes.

Levels of progesterone >3 nmol/L were considered as indicative of the presence of a CL, while levels <1 nmol/L were considered indicative of the absence of luteal function. Intermediate levels (1–3 nmol/L) were considered inconclusive [7]. The progesterone concentration at the three stages of sampling was used to deduce the reproductive status of the cows, timeliness if AI and the outcome. The conclusions from the progesterone assay were eventually compared with results from pregnancy diagnosis done by rectal palpation between days 60–75 after AI.

The data collected relating to the farm, cow, heat period, inseminator and semen batch were subjected to contingency analysis to determine the effects of these factors on CR. Seasonal effects were also assessed from January to March, April to June, July to September and October to December, which were differentiated based on average rainfall. The mean rainfall for these periods was 107, 255, 213, and 180 mm, while the mean ambient temperatures for the same periods were 28.6, 26.2, 25.4 and 25.8°C respectively.

A total of 2500 milk samples were assayed from 952 cows. However, not all cows had the full set of three samples and, for the purposes of this paper, only the data from 481 cows that had results for all three samples are presented.

3. RESULTS

The breed distribution of the cows from which all three samples of milk were available for progesterone measurement is given in Table II.

TABLE II. BREED DISTRIBUTION OF COWS FROM WHICH THREE SAMPLES OF MILK WERE ASSAYED FOR PROGESTERONE

Breed	No of Cows	% of Total
Aryshire	49	10
Friesian	401	83
Guernsey	15	3
Jersey	16	4
Total	481	100

Based on the samples assayed and subsequent rectal palpation, 255 cows (53%) were diagnosed pregnant. The progesterone concentrations determined at the three stages of milk sampling for these cows are presented in Table III.

TABLE III. MEAN PROGESTERONE LEVELS AT THREE STAGES AFTER AI IN COWS SUBSEQUENTLY DIAGNOSED BY RECTAL PALPATION AS BEING PREGNANT (N=255)

Days after AI	Progesterone Concentration (nmol/L)			
	Minimum	Maximum	Mean±SE	Median
0	0	7	0.43 ± 0.02	0
13	0	15	5.31 ± 0.18	5
23	0	26	10.06 ± 0.26	10

For the majority of cows that were not pregnant at palpation (N=226), progesterone levels were depressed at all stages of sampling when compared with those in pregnant cows. The mean levels were 0.59 ± 0.09, 2.69 ± 0.17 and 0.46 ± 0.04 nmol/L on days 0, 13 and 23 after AI, respectively. The numbers of cows for which the progesterone concentrations were <1 nmol/L or >3 nmol/L at the time of AI and the subsequent pregnancy diagnosis based on rectal palpation are shown in Table IV.

TABLE IV. PROGESTERONE CONCENTRATION AT TIME OF AI AND RESULTS OF SUBSEQUENT PREGNANCY DIAGNOSIS

Progesterone (nmol/L)	Number of Cows	%	Pregnancy Diagnosis
<1	217	45	Pregnant
<1	185	38	Non-Pregnant
>3	38	8	Pregnant
>3	41	9	Non-Pregnant

Thus 83% of the cows were inseminated at the right time, that is when the progesterone levels were low, but 46% of these did not conceive. Seventeen percent of the cows were inseminated when progesterone levels were high, indicating that they may have been in the luteal phase, but 8% of them were found to have conceived, and may have been inseminated while already pregnant.

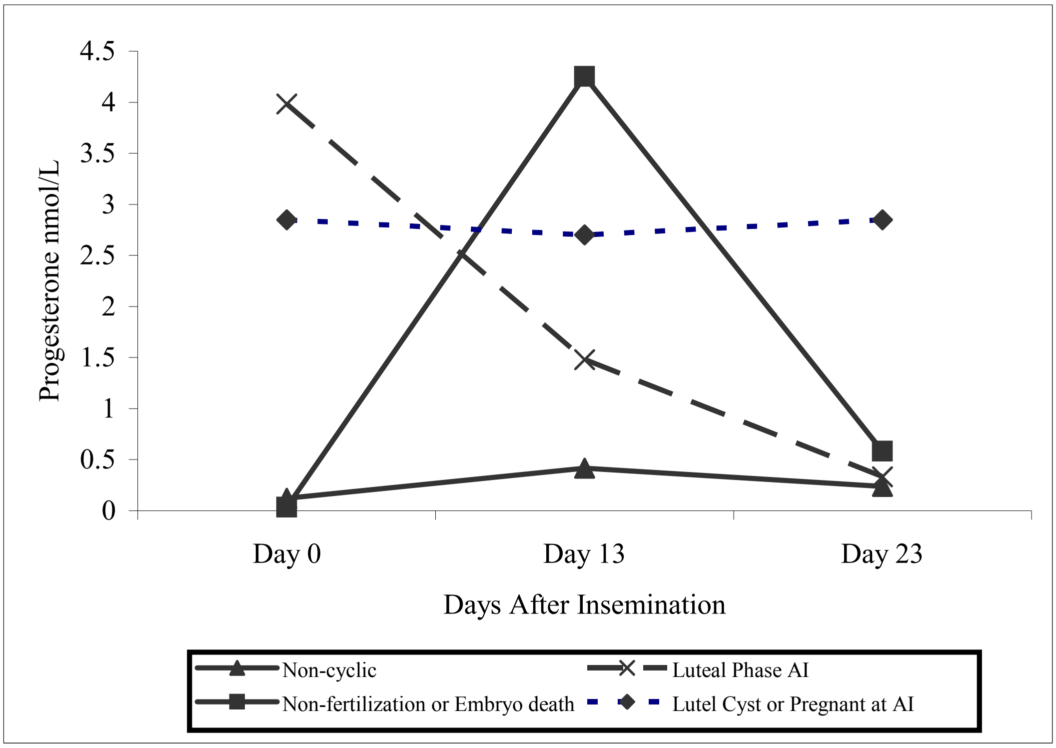

FIG. 1. Progesterone profiles for non-pregnant cows.

Figure 1 shows the typical progesterone profiles for groups of cattle in which AI was not successful. Based on these profiles, it was estimated that non-fertilization and early embryonic mortality accounted for 54% of the cows diagnosed not pregnant, while 32% had low progesterone concentrations at all three stages, indicating that they were non-cyclic. Elevated progesterone was found at the time of AI in 14% of the non-pregnant cattle, indicating that they were inseminated either during the luteal phase or while pregnant.

The range and mean values for the intervals from calving to first AI and from the first detection of heat to AI are shown in Table V.

TABLE V. INTERVALS FROM CALVING TO FIRST INSEMINATION AND FROM DETECTION OF HEAT TO AI

Interval	Minimum	Maximum	Median	Mean ± SE
Calving to first AI (months)	1	12	4	4.8 ± 0.15
Detection of heat to AI (hours)	4	26	21	19.2 ± 0.33

A contingency analysis of CR by breed, season, time of AI, site of AI and inseminators indicated that cows of different breeds did not show significant differences (P >0.05) for conception. However, as shown in Figure 2, there were significant differences (P <0.05) in the numbers of cows inseminated and the CR during different seasons. Most inseminations occurred during the October to December (short rain period), while the highest conception rates were recorded during April to June (long rain period). The time of AI (AM or PM) and site of semen deposition (vagina, cervix or uterus) did not have significant effects on CR.

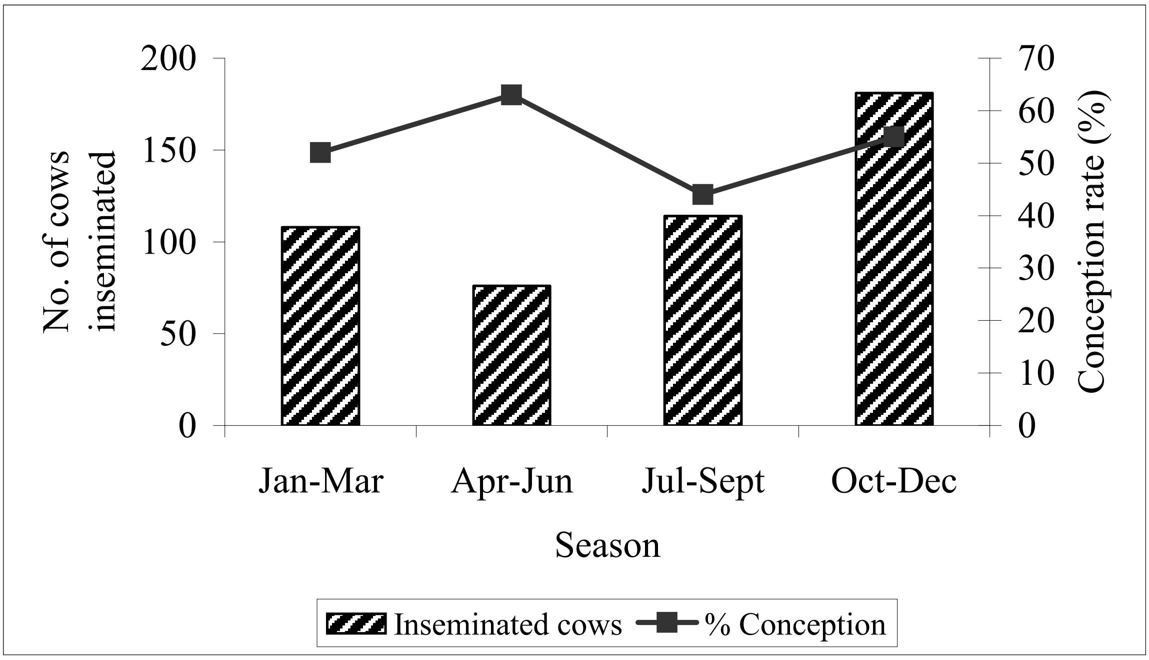

FIG. 2. Effect of season on number of AI service and conception rate.

TABLE VI. ACCURACY OF PREDICTION AND DETECTION RATE FOR PREGNANT (P) AND NON-PREGNANT (NP) COWS BY USING PROGESTERONE (P4) MEASUREMENT 23 DAYS AFTER AI

Parameter			Rate (%)
Accuracy of predicting NP	No. with low[a] P4 = 233	No. confirmed NP = 224	96%
Accuracy of predicting P	No. with high[b] P4 = 248	No. confirmed P = 246	99%
Detection rate for NP	Total NP cows = 226	Of the NP cows, those with low P4 = 224	99%
Detection rate for P	Total P cows = 255	Of the P cows, those with high P4 = 246	96%

[a] Progesterone <3.2 nmol/L; [b] Progesterone >3.2 nmol/L

Table VI shows the accuracy of the progesterone assay for predicting non-pregnancy and pregnancy on day 23 after AI, and the detection rate of this method for cows that were subsequently found to be pregnant or non-pregnant.

4. DISCUSSION

The breed distribution of the cows that were sampled during this study is a reflection of the breed preference by farmers in this region of the country, with 83% of the farmers preferring the Friesian because of its perceived high milk production. The numbers of Jersey and Guernsey cattle are declining even though they have lower management and feed requirements compared with the Friesian, and would therefore seemingly be more suitable for the small-scale production system of this region. Further, although these Channel Island breeds have higher butter fat content in the milk than Friesian, the price of milk in Kenya is not dependent on butterfat content. Although the Ayrshire breed is quite popular in some regions of the country, it was a distant second in the present study, the results of which agree with previous survey results by Bebe *et al.* [8].

In the cows that conceived to AI, the progesterone levels were basal at the time of AI (day 0) and showed a steady increase through days 12 and 23, indicating the development of an active CL. This result agrees with the findings of McLeod *et al.* [9] who observed increased conception rates when basal progesterone levels were almost zero at the time of AI, followed by a progressive rise.

The finding that 83% of the cows were inseminated when progesterone levels were low indicates that the efficiency and accuracy of oestrus detection was high among the farmers participating in this study. However, 32% of these cows did not show a subsequent elevation of progesterone on day 12, indicating that they were non-cyclic. Of those that did have elevated progesterone 12 days after AI, 38% were found to be non-pregnant, which represents a high percentage of reproductive wastage due to either non-fertilization or early embryonic loss. Larson et al. [10] found that reduced progesterone levels post breeding could reduce fertility, but the sampling frequency used in the present study was inadequate to compare the values at several stages after AI in those that did or did not conceive.

Seventeen percent of the cows were inseminated when progesterone levels were high, indicating that they had an active CL at the time and that the farmers misdiagnosed oestrus. Nearly half of these cows were later found to be pregnant, indicating that they had been inseminated during pregnancy. The others were most likely in the luteal phase when inseminated [6].

The timing of insemination after heat detection is crucial in order for fertilization to take place, since delay will result in the ovum being aged by the time capacitated sperm reach the oviduct. Our data showed a mean interval of 19 hours from heat detection to insemination, which is in accordance with the recommendations of Hafez and Hafez [6] that cows should be inseminated 12 to 18 hours after onset of oestrus. Significant differences for CR ($p < 0.05$) were observed among the different inseminators. This variability could be attributed to differences in skills, competence and commitment.

The interval from calving to first AI was 5 months in the present study, which is much longer than the ideal of 2 months in order to obtain a one year calving interval. This time lag was the main reason for long calving intervals in the farms under study and could be attributed to factors such as low nutrition resulting in anoestrus, poor heat detection by farmers and physiological dysfunction of the cows leading to non-conception after AI.

The seasonal influence observed on heat expression and the subsequent CR could be mediated through environmental attributes like rainfall, which directly influence herbage quantity and quality, and result in the higher CR seen during the long rain season from April to June. The high number of cows expressing heat during October to December could be due to the optimum ambient temperature and herbage availability during the short rain period. The improved nutrition improves body condition, which is necessary for heat expression and improved conceptions.

Embryonic mortality and non-fertilization after AI accounted for more than 50% of the non-pregnant cows. The main reasons for this occurrence could be attributed to nutritional stress, which has been shown to lower ovulation rates in sheep as a result of decreased LH pulse frequency [11]. In cattle, Sreenan and Diskin [12] have reported that early embryonic losses occur in about 38% of all conceptions, mostly between days 15 to 18 after AI. On the other hand, anoestrus or acyclicity also plays a significant role in the failure of cows to conceive after AI [13].

In conclusion, the main reasons for non-pregnancy in the present population of cows were non-cyclicity, non-fertilization (wrong time of insemination and presence of luteal tissue) and early embryonic mortality. The use of progesterone measurement for early diagnosis of non-pregnant cows has a high level of accuracy and can improve the efficiency of AI delivery, reduce calving intervals and improve milk and meat production from the dairy industry. The next crucial step is to develop a simple, accurate and affordable on-farm testing method that can be used by the AI technicians or the farmers themselves. This will overcome the logistic problems of getting AI technicians and farmers to bring or send milk samples to a central laboratory, which is often far away from the farms. Development of such a 'cow-side' test will clearly enhance the use of AI among dairy farmers and result in improved economic gains for them as well as increased milk and meat production on a national scale.

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REPRODUCTIVE CHARACTERISTICS AND HERD DYNAMICS IN SMALLHOLDER DAIRY FARMS OF NAKURU, KENYA

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Abstract

A survey was conducted to quantify cattle reproduction characteristics and herd dynamics in five divisions of Nakuru District. A cross-sectional stratified random sample of 60 households (12 farmers in each division) was selected and a semi-structured questionnaire was administered. Cross tabulation frequencies and non-parametric analysis were used to analyse the data. The land holdings ranged from 2.5 to 9 acres and the maximum distance between any locality the nearest town was 33 km. The mean household size was 5 persons and 82% of the farmers had at least primary education. Cattle were the preferred species and 52% of the farmers kept at least 4 head of cattle. The production systems were 48% zero grazing, 42% semi-zero and 10% extensive. The breed distribution was 45% Friesian, 37% Ayrshire and 6% Guernsey and Jersey. Family labour contributed 58% of the farm labour and women managed 33% of the farms. At the time of the survey only 28% of the cows on the farms were pregnant and more than half of the mature cows were not lactating. Around 47% of the calves born left the herd before weaning and heifers formed only 12% of the existing herd, making it necessary to buy replacements when culling occurred. The average milk production was 8.5 litres per cow per day. Most of the farmers used AI for breeding and 62% of the cows required four or more repeat inseminations before conception. Sixty-nine percent of the farmers used non-return rate for determining pregnancy, resulting in long calving intervals and fewer calves born. Rectal palpation was used in 12% of farms and was done about 90 days after breeding. It was concluded that reproductive efficiency can be increased through improved nutrition and heat detection, and provision of a service for early non-pregnancy diagnosis.

1. INTRODUCTION

Kenya has been one of the few countries in Africa to support smallholder dairy development successfully [1]. Smallholder farms account for between 75 to 90% of all milk produced in Kenya [2]. However, most of the increased production in the smallholder sector has been due to extended use of land and livestock resources rather than from higher individual cow productivity [1]. Pressure on land due to population expansion will naturally require that smallholders intensify dairy production. Such intensification will require improved management and increased resources per cow if it is to be sustainable [3]. However, improvement of this sector will result in large social benefits because of the large labour force available and the commitment of farmers [4].

An important constraint to dairy production is low nutrition, this factor plays an important role in limiting the average milk yield to only 5.8 kg per cow per day [5]. The second major constraint is impaired fertility in cows, resulting in long calving intervals, which average 633 days [6]. On a herd basis, a shorter calving interval results in greater milk production per day and more calvings per year. In systems practicing artificial insemination (AI), the length of the calving interval is influenced by the time interval to the first insemination, the number of inseminations per conception and the number of days open from calving to conception [7]. Under normal grazing conditions in the Nakuru District, the average number of services per conception has been reported to be 2.5 [8]. Friesian and Ayrshire are the predominant breeds in these smallholder units [9, 10]. These breeds are among the highest producing dairy breeds world-wide, but are larger than other breeds and, therefore, have a greater demand for maintenance and production than, for example, the Guernsey and Jersey, which are the ones technically recommended, considering feed and other management constraints [3].

The objectives of this study were to collect baseline information on smallholder dairy systems of Nakuru District and to determine the challenges that impair reproduction, with a view to determining interventions that can address the constraints.

2. MATERIALS AND METHODS

A survey was conducted to quantify cattle reproduction characteristics and herd dynamics in five divisions of the Nakuru District. The divisions selected were Gilgil, Nakuru, Njoro, Olkalau and Subukia, and were chosen on the basis of their participation in an on-going project for early non-pregnancy diagnosis using milk sampling and measurement of progesterone concentrations with radioimmunoassay. This project is carried out in conjunction with community-based organizations (CBO) that provide AI services to farmers. A cross-sectional stratified random sample of 60 households (12 farmers in each division) was selected and the survey was done during the month of May 2004.

Data collection was done through household interviews conducted by trained enumerators using a pre-tested semi-structured questionnaire. The information gathered included household characteristics with emphasis on levels of education, family size, labour and land use; and farm characteristics including farm size, number of livestock by class and grazing systems. The herd demographic data, including births, purchases, deaths and sales, were recorded based on the respondent's recall of events for the year 2003. Information on reproduction and fertility formed the main body of the questionnaire, including utilization of AI services and its costs, methods of pregnancy diagnosis, challenges and opportunities.

In each division, approximately 12 questionnaires were administered to the dairy farmers within a period of 4 days. The enumerators were AI practitioners of the community based organizations that were operating in the respective divisions. When, needed assistance was provided by members of the staff from the Kenya Agricultural Research Institute (KARI) at Nakuru, Naivasha and Nairobi. Sixty households fully answered the questionnaires.

The data collected was entered into SPSS version 8 databases for statistical analysis. Simple descriptive statistics were calculated and cross tabulation and frequencies of occurrences were used to characterize the data, while non-parametric tests were used to evaluate and prioritize the reasons why farmers used different practices.

3. RESULTS

The characteristics of households and farms sampled in the five divisions are given in Table I. The majority of respondents were owners of the farms and 66% of them were males. The level of education of the respondents is given in Figure 1. Farm activities were carried out by family members, who provided 58.5% of the labour requirements.

TABLE I. CHARACTERISTICS OF THE HOUSEHOLDS AND FARMS SURVEYED

Division	Number of households	Mean size of household	Mean land area (Acres)	Distance to nearest town (Km)
Gilgil	15	5	8	5
Nakuru	7	5	2.5	3
Njoro	15	6	9.5	17
Olkalau	12	5	5	4
Subukia	11	6	5	33

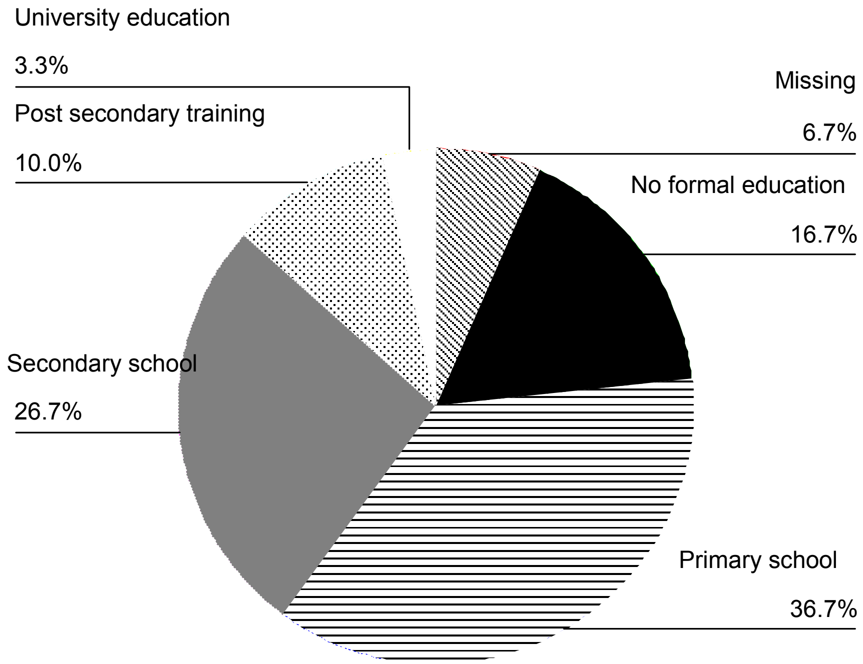

FIG. 1. Literacy levels of the farmers interviewed

The farmers interviewed kept 513 cattle (Table II), 242 sheep, 154 goats, 1256 grade chicken and 697 indigenous poultry. Most of the sheep were found in Subukia, Olkalau and Njoro, while grade chickens were mainly found in the Gilgil area, particularly in farms bordering the Bahati area.

TABLE II. NUMBER OF CATTLE KEPT BY THE FARMERS AND PERCENTAGES OF DIFFERENT CLASSES IN THE FARMS SURVEYED

Division	No of cattle	Lactating cows (%)	Dry cows (%)	Weaners (%)	Heifers (%)	Calves (%)	Bulls (%)
Gilgil	115	35	38	4	9	11	3
Nakuru	106	30	37	8	12	13	0
Njoro	147	24	39	11	13	10	3
Olkalau	77	30	39	3	7	9	5
Subukia	68	24	43	6	15	9	4
Mean (%)		29	39	6	11	10	3

The distribution of different breeds of cattle in the surveyed divisions is given in Table III. The mean milk production for cows was about 8.5 liters per day (Table IV). The total number of calves born in 2003 was 117. Considering that there were 346 mature cows, the overall calving rate in the herds was 35%.

Out of the calves born during the year, 47% had left the herd before weaning and heifers formed only 17% of the existing female population.

TABLE III. DISTRIBUTION (NUMBER) OF CATTLE OF DIFFERENT BREEDS IN THE FARMS SURVEYED

Division	Friesian	Aryshire	Jersey	Guernsey	Crosses
Gilgil	62	30	6	11	17
Nakuru	41	34	0	6	4
Njoro	41	41	6	6	11
Olkalau	47	51	6	0	6
Subukia	25	30	16	6	6
Mean (%)	43	37	7	6	9

TABLE IV. MEAN MILK YIELD/COW/DAY AND CALVING RATES IN THE FIVE DIVISIONS

Division	Milk yield (Litres)	No. of calves born	No. of mature females	Calving rate (%)
Gilgil	9.5	23	84	27
Nakuru	10.5	19	71	27
Njoro	7.5	32	92	34
Olkalau	9.5	23	53	43
Subukia	6	20	46	43

The majority of farmers (69%) relied on ‘non-return’ to heat as the method for determining whether a cow had become pregnant to AI. Twelve percent of farmers had their cows examined for pregnancy by rectal palpation, while 7% submitted milk samples for measurement of progesterone by radioimmunoassay and the reminder used a combination of these three methods.

Only 6% of the cows conceived after the first service, while 13% required two and 19% required three services. The remaining 62% required 4 or more inseminations. The overall percentage of mature cows that were found to be pregnant at the time of the survey was 29 (22% in Gilgil, 18% in Nakuru, 31% in Njoro, 41% in Olkalau and 31% in Subukia).

4. DISCUSSION

The mean land area of the farms ranged from 2.5 to 8 acres and was related to the proximity to main towns, with rural divisions having larger farms compared to those near the urban centres. This was expected, as available land diminishes with urbanization, and the findings are similar to those of a previous study in Nakuru [3] where average farm size was 4 acres. The farm size appeared to influence the type of land use, with 10% of the farmers practicing extensive cattle production, 42% semi-zero grazing and 48% zero-grazing systems.

Households averaged 5 persons, with rural divisions (e.g. Subukia) having slightly more people, where most of the farm activities are carried out by family labour. In comparison, urban divisions (e.g. Nakuru and Njoro) had smaller households and their members were engaged in waged employment, resulting in the use of hired labour to perform farm chores.

The distance to the nearest town can impact productivity because of infrastructure problems affecting access to markets and availability of services like AI and farm inputs. This directly influences the development of the dairy sector. The low literacy levels recorded in Subukia could be attributed to its rural setting, being 40 km away from Nakuru town. This has direct implication on adoption of technology and improvement of productivity, with divisions having lower literacy levels recording lower mean milk yield. However, 76% of the farmers had at least primary education or above and should be able to comprehend the importance of adoption of technology for increased productivity. The 16% illiterate farmers should be able to learn from the majority of the enlightened farmers, thereby contributing to faster rural development.

On average, each household had 8 head of cattle with an average land holding of 6 acres, giving a carrying capacity of 1.3 head per acre. Looking at the herd structure, it was evident that 68% of the cows were mature, but 57% of them were not in milk. This indicates that the potential for milk production from these enterprises was not being realized [10], because these cattle have to be fed and maintained without producing. Heifers accounted for only 12% of the herd, which indicates that the farmers are not able to replace the animals that are disposed of at an annual rate of 24% [3]. Therefore, farmers have to continually buy in stock in order to maintain the herd size.

Bulls were kept by farmers in Subukia and Gigil and formed 3% of the herd. These were mainly kept in the remote areas where AI is not readily available. Proportionately, Njoro had more livestock than other regions due to good livestock services and infrastructure.

Friesian was the breed of choice, with the highest proportions being reared in Njoro and Gilgil divisions. The high proportion of Friesians in Njoro and Gilgil could be attributed to the presence of two major Friesian breeders, namely Delamere and Susumwa Estates, in these two areas, resulting in availability of breeding stock for purchase by smallholder farmers. It was found that farmers tend to prefer Friesians because of their expected higher milk yield as well as the higher price fetched by culled animals in comparison to other breeds. However, the majority of Friesians kept by the farmers were not in good body condition, mainly due to the inadequate feed provided to them [12]. The low milk yield obtained from them also confirmed that the full potential of the breed was not being realized due to poor feeding and management. Although the Guernsey and Jersey have been suggested to be the most suitable breeds for the level of management and feeding available under the smallholder production systems [3], their numbers have declined.

At the time of the survey, only 29% of the cows were pregnant, confirming the low annual calving rate, which was calculated to be 35%. The conception rates ranged from 18% in Nakuru to 41% in Olkalau. The majority of farms had a high level of infertility, which leads to reproductive wastage [6]. This problem could be due to a host of factors, including poor heat detection, improper timing or technique of insemination, poor semen quality and inadequate body condition of the cows. As a consequence, cows required many services before they became pregnant and had long calving intervals, resulting in excessive days open and long, unproductive dry periods. It also means that the herds are not able to generate their own replacements.

With 69% of the farmers relying on non-return to heat as an indicator of pregnancy, it is likely that a major reason for the low conception rates recorded was poor heat detection. The availability of people to observe heat during the critical periods is usually a problem and most heats probably go unnoticed. The alternative method of rectal palpation is considered expensive and is usually done at least 60–75 days after AI, leading to lost breeding time and long postpartum periods. This lost opportunity in turn results in fewer calves born, reducing the revenue that can accrue from the sale of milk and surplus calves, and also impairs genetic gain due to the fewer calves from which selection can be made for replacements.

The results from this study point to an overwhelming need for technological interventions to overcome the low levels of fertility in these herds, which result in low production of milk and calves. Reproductive efficiency can be increased through improved nutrition and heat detection, and provision of a service for early non-pregnancy diagnosis.

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ANALYSIS OF THE COST AND BENEFIT OF BOVINE ARTIFICIAL INSEMINATION IN SENEGAL

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Abstract

A field survey was conducted to determine the rate of success of artificial insemination (AI) in cattle in Senegal and the benefit obtained by raising the resulting F1 crossbred heifers. The area selected was the first one in which a public AI programme was implemented. A questionnaire was administered to farmers regarding the management of cattle and the costs and benefits of the AI service. Out of 207 inseminations, 80 were successful, resulting in a calving rate of 38.6%. The F1 crossbreds produced 8.9 litres of milk per day, compared with 2 litres per day for the local breeds, and had a lactation length of 13 months. The death rate for crossbred calves during the first year of life was 22.5%. The total cost for an AI was FCFA 48 143 per cow, and was mostly subsidized by the Senegal State. The net profit for the producer was FCFA 35 (Euro 1 = approx. FCFA 660) per litre of milk produced by a crossbred. The proportional costs of the different components of the AI service were 12.5% for the purchase and conservation of semen, 21.9% for the synchronization of heats and 53% for provision of the AI service. The study identified weaknesses and limitations in the dairy production system. Future actions are recommended to improve the results of AI, to reduce the costs and to increase the benefits to producers.

1. INTRODUCTION

The production of milk and meat of local cattle breeds in Senegal is very low in comparison with that of the breeds of animals which exist in the developed countries. Daily milk yield does not exceed 3 L per cow, and the duration of lactation is only about 5 months. To meet the needs of the human population, milk and meat are imported from developed countries. Since the devaluation of the local currency, studies show that Senegal has spent more than 30 billion FCFA (current exchange rate is approximately Euro 1 = FCFA 660), to support milk importation.

In 1995 a national programme was launched in order to increase the production of milk and meat by genetic improvement of the local breeds. Artificial insemination (AI) is an important technique that makes it possible to carry out this genetic improvement. The advantage of this technology is that more intense genetic selection can be practiced, because a relatively small number of genetically superior bulls can inseminate many cows. It can also be used to control venereal and contagious diseases. However, an important consideration is that conception rate should not be adversely affected by the use of AI.

Several factors influence the reproductive performance of dairy cows when AI is used. They include the precise detection of oestrus, the correct timing of insemination, the quality of semen used and the skill of the inseminator. Other factors related to the management, feeding and health of the cows also influence the final outcome. Studies were therefore undertaken to obtain information on the management of reproduction in small-holder livestock farms, together with the measurement of progesterone levels in blood and milk using nuclear-based methods. The overall objective was to establish the level of fertility in the herds with an aim of managing the administration of AI and to determine the relationship between the characteristics of the cows and the conception rate to the first service.

The specific objectives of the study were to: (a) calculate the rate of success of AI; (b) determine the costs relating to the insemination and additional feed for the F1 offspring; (c) compare the productivity of the F1 crossbreds with that of the local purebreds; (d) calculate the net profit per litre of milk at the level of the individual producer and at the level of the production system; (e) evaluate the impact of bovine AI in Senegal; and (f) evaluate and improve the AI programmes in the traditional small-scale dairy farms.

2. MATERIALS AND METHODS

All the studies were undertaken in close cooperation with other relevant institutes; including the Senegalese Institute of Agronomic research (ISRA), the Office of Livestock Breeding in Senegal (DIREL) and the Project for Support to Breeding (PAPEL).

2.1. Location and subjects of the study

The studies were performed under two types of dairying systems, extensive and intensive. Extensive dairying refers to a system based entirely on pasture (free grazing system), where fodder crops are generally not raised and herds are usually made up of several species (bovines, sheep and goats) which may be mixed or maintained separately. Extensive systems are practiced in two forms: pastoral and agro-pastoral.

The intensively managed dairy farms in Senegal are generally localised in the peri-urban zone of Dakar (the capital), and are the properties of private owners. These farms are characterized by high standards of technical and economic management with intensive production for purely dairy purposes. The cattle are usually of exotic breeds and have been subjected to AI for several years.

The study included AI organizations and farms (producers) that were both private (PROELES, GIE Cap Vert, AFRIVET) and state owned (DIREL, PAPEL). Most of the inseminations were performed after synchronization of oestrus in the cows.

2.2. Questionnaire

A questionnaire was used as an adapted means of data acquisition. It contained tables and questions, some of which were open to allow best possible options and opinions to be recorded.

2.3. Sampling

Sampling was based on a reasoned choice. For more convenience, the targeted farms were those that were included in the sampling of the Concerted Project of Research-development (PROCORDEL), within the framework of their follow-up programme of the crossbreds in the area of Fatick. In total samples were provided from 21 farms.

2.4. Statistical analysis

After coding and examination, the data collected were captured on Excel spreadsheets and were processed with statistical software SPSS (Statistical Package for the Social Sciences) for the descriptive statistics. The frequencies, the sample means and the standard deviations were calculated.

2.5. Economic analysis: partial budget analysis

The objective was to see whether the increase in the milk production or income obtained by the use of AI constituted an adequate or at least satisfactory remuneration for the increased effort and expenditure made by the farmer. This analysis of the partial budget was based on three important assumptions: (a) the whole sample is regarded as only one manufacturing unit; (b) a standard daily feed ration is used; and (c) the inseminated local cow gives rise to a viable female crossbred and the additional expenses relating to the feed start with the birth of the crossbred.

The aspects evaluated were: (a) the additional benefit which can result from an increase in the production or income and a reduction in the production costs; and (b) the extra costs which correspond to an increase in the expenditure or a reduction in the production or income. The net benefit or profits were simply calculated as the difference between the additional benefit and the extra costs.

3. RESULTS

3.1. AI service: rate of success

The 21 farms surveyed were primarily distributed in the departments Fatick and Foundiougne. The number of cows inseminated per farm was variable from one year to the other and ranged from 2 to 5. A total of 207 inseminations were recorded in the sampled farms during the period 1996 to 2001. The cost borne by each producer per inseminated cow was variable across years and ranged between FCFA 4000 to 10 000, with an average of FCFA 7367 ± 1867.

The diagnosis of gestation by per rectal palpation was usually done 3 months after insemination by the technicians. The success rate of AI was taken as the calving rate, which was 38.8% (80 calves born from 207 inseminations). The offspring were mainly F1 crossbreds, including both males and females.

3.2. Reproductive management by farmers and reproductive parameters

The management of F1 crossbred cattle was based on the integrated use of pasture and supplementary feed, with the ration primarily consisting of roughage, concentrates and salt. The precise ingredients and quality of a "standard" ration was very difficult to characterize because of the disparity of the rations from one farm to another. For the financial analysis, the price of a standard ration fed to F1s was calculated with the assumption that the whole sample is only one manufacturing unit. The estimated proportions of ingredients in the standard ration were based on their overall frequency of use.

As shown in Table I, the average cost of the ration for F1 crossbreds was FCFA 860 per day. The farmers did not have adequate infrastructure such as housing, equipment and related materials for improved cattle husbandry. The cattle sheds were usually constructed of local materials such as wooden pillars and a roof thatched with straw, or in some cases no roof. The feeding troughs and mangers were usually made by cutting barrels in two. The introduction of the F1 crossbreds did not prompt farmers to upgrade or otherwise modify the housing of their animals.

TABLE I: STANDARD DAILY FEED RATION FOR CROSSBREDS AND THE COST PER COW

Ingredient	Quantity (kg/day)	Price/kg in FCFA	Total Price in FCFA	Percentage cost
Fodder (pasture and straw)	4	20	80	9.3
Jarga (concentrate of cereal bran)	3.61	165	596	69.3
Millet bran	2.45	75	184	21.4
Total	10.06	-	860	100

3.3. Production parameters

Just as for the feed ration, the dairy production was variable from one farm to another. The average quantities of milk produced per day were 9 L for the F1 crossbreds compared with 2 L for the local breed. The increased quantity of milk produced by crossbreds was also associated with longer lactations, averaging 12.7 months (Table II). The selling price of milk varied between FCFA 250 and 350 per L, with an average price of FCFA 281. The F1 crossbreds grew quickly when management and nutrition were sufficient and reached breeding age at an average of 18 months. In addition, compared to the local breed, the selling prices of the F1 crossbreds were much higher, with a 15 month old crossbred costing approximately FCFA 200 000 (Table III).

TABLE II. MILK PRODUCTION OF F1 CROSSBREDS AND LOCAL COWS

Variable	n	Minimum	Maximum	Mean	SD
Lactation length of local cows (months)	20	6	11	9.1	2.3
Lactation length of F1 crossbreds (months)	9	10	15	12.7	1.8
Milk production of local cow (L)	19	1	4	1.9	0.9
Milk production of F1 crossbreds (L)	9	6	14	8.9	2.2

TABLE III. AGE AND AVERAGE PRICE OF F1 CROSSBREDS AND LOCAL COWS

Age (months)	Price in FCFA	
	F1 Crossbred	Local Cow
15	200 000	80 000
24	250 000	130 000
36	300 000	150 000

3.4. Economic analysis

The extra costs due to the use of AI included the payment by the farmer for the AI service and extra inputs for feed and anti-parasitic treatment for F1 crossbred offspring. The foundation breeding stock of local cows was considered as not having any monetary value. Similarly, there were no specific health problems with the F1 crossbreeds and no extra costs were incurred due to diseases.

Costs for feed were calculated on the basis of average cost of the feed ration per day (Table I). The average reproductive age of the female F1 crossbreds was 18 months. One production cycle was considered as starting from the date of birth to the end of the first lactation. The duration of this cycle was therefore 38.7 months (1191 days). The real extra cost to the producer for each inseminated cow resulting in a female calf was FCFA 19 065 and the total additional cost was FCFA 1 043 326 (Table IV).

The difference between the milk production obtained from local and F1 crossbred cows was taken as the additional income from milk and the difference between the selling price of local and F1 crossbred cows at 40 months of age was taken as the additional value for the animal. The net profit or net benefit drawn by the manufacturing unit with the use of insemination was therefore FCFA 85 199 (Table VI). The net profit reported per litre of milk was FCFA 35.

This calculation was based at the micro-economic level, which is the scale of the producer. In order to make the calculation on a broader scale, at the macro-economic level or system of production, data were collected on the actual costs of AI, some of which are subsidized by the Senegalese State. The information obtained from various private AI service providers and official organizations are summarized in Table V. The components that are subsidized include the cost of importation of frozen semen, synchronization hormones, equipment for semen conservation and AI, and part of the payments to the service providers for the inseminations and follow-up of the cows. The net profit from an AI service calculated at the scale of the system of production was FCFA 69 203 per F1 crossbred cow and FCFA 20 per L of milk.

TABLE IV. ADDITIONAL COST, INCOME AND NET PROFIT FROM ONE F1 CROSSBRED CALF WHICH COMPLETES THE FIRST LACTATION.

Additional cost	FCFA	Additional Income	FCFA
Insemination	19 065	Value of extra milk produced	808 524
Feed ration	1 024 260	Value of the F1 crossbred	320 000
Total	1 043 325		1 128 524
Net profit			85 199

TABLE V. CONRIBUTION OF THE DIFFERENT COMPONENTS OF THE AI SERVICE TO COSTS

Component	Cost in FCFA	Percentage
Semen	6 000	12.5
Synchronization of heats	10 568	21.9
Semen conservation	5 000	10.4
Depreciation of AI equipment	1 075	2.2
Payment to service providers	25 500	53
Total per cow inseminated	48 143	100

3.5. Health and mortality

There were no major health problems reported in 76% of the farms surveyed. The remaining 24% of the farmers reported having cases of dermatitis (skin infections) and liver fluke in their herd. However, it is important to note that these diseases were not specific to the F1 crossbreds. The disease prevention activities in this region were limited to the national campaigns of vaccination against pasteurellosis and bovine contagious pleuropneumonia.

In the whole sample, 18 cases (22.5%) of mortality of F1 crossbreds were reported between 0 and 12 months of age.

4. DISCUSSION

4.1. Descriptive analysis

The calving rate obtained from AI in our study was 38.6%, which was lower than the rate of 43.4% obtained over a four-year period by the state organization PAPEL. This result is similar to that obtained (38%) in Burkina Faso by the National Programme for Control of Dairy Development and higher than that obtained (24%) in zebu cattle of the Azawak breed [1]. It must be noted that most of the inseminations in our study were done after synchronization of oestrus. Thus, the protocol of oestrus synchronization as well as the methodology for the AI may not have been optimal. In Cameroon, with AI after natural oestrus, a calving rate of 42.5% has been reported [2]. Other reasons for the low calving rate in our study could be due to poor knowledge of the farmers regarding the reproductive and physiological states of the females, poor nutrition and lack of adequate disease control.

The most important factor in the success of AI is nutrition. In our area of study, the nutritional status of the animals is largely determined by the quality and availability of fodder. The average quantity of fodder consumed per crossbred animal was 4 kg per day. On the other hand, previous reports have indicated that a crossbred may need around 8.6 kg per day [3]. The availability of the forage is strongly influenced by rainfall and other factors such as the reduction of pastoral land area due to extension of cultivation and salinisation. In addition, transhumance of cattle from the neighbouring localities such as Diourbel influence fodder availability [4]. Although the crossbreds in our study received concentrates as supplements to fodder, the purchase of ingredients for the concentrate feed was a major constraint faced by many farmers due to the high cost.

The second important factor is the control of diseases and mortality, especially in young calves. In our results, the death rate of 22.5% for calves up to one year of age is much higher than the rate of 5.2% obtained by the PAPEL in 1999. In Mali, a rate of 8.7% was reported for calves up to 6 months old [5]. This mortality can be explained by poor hygiene and disease control, inadequate care at time of birth and adverse effects of climate. In our study, no specific health problems were detected that were unique to the crossbreds. However, previous studies in the area of Basin Arachidier of Senegal found that mastitis occurred on 33% of farms [3], while studies in Mali found that 90% of crossbred herds had problems due to parasitic diseases [6].

The average duration of lactation for the crossbreds was 12.7 months and the milk production was 8.9 L per day per animal. Other authors reported a higher production with an average of 12–14 L per day for Jersey x Ndama crossbreds and 18–19 L per day for the Montbéliarde x Gobra crossbreds from peri-urban farms [7].

4.2. Economic analysis

The net profit per litre of milk resulting from a crossbred produced by AI, calculated at the scale of the producer, was FCFA 35. This value lies within the range of net profit found in Burkina Faso, which was FCFA 3–106 [8], but is higher than that obtained in 1999 by the PDAP, which varied from FCFA 2–31. It should be emphasized that the method of calculating net profit may have varied between studies. In our study, factors such as mortality, the sex ratio and the extra costs relating to the food of the inseminated local cow were not taken into account. However, it is clear that the profit could be improved by an increase in the rate of success of AI and in the milk production.

The cost of AI in our study was relatively high, due mainly to the high costs in synchronization of oestrus and provision of the AI service. The average price of the semen was FCFA 6000 and represented only 12.5% of the cost of an AI. However, this was high when compared with the price of FCFA 3422 reported in Mali [6]. It should be noted that the price of the semen varies according to its quality. The cost of synchronization of heats was also dependent on the procedure used. A previous study found that the cost was lower, being FCFA 6270, when Progesterone Releasing Intravaginal Devices (PRID) were used and FCFA 6290 when ear implants (Crestar) were used [9]. Doing AI during natural heats would significantly reduce the cost, but its use is limited due to poor heat detection by the farmers and the logistics of getting the inseminator to the cows at the correct time. The cost of providing AI services was also higher than that reported in Mali [10], which was FCFA 10 615.

In conclusion, this study has highlighted some deficiencies which the dairy industry must address in order to improve the goals of production and economic benefits. A better organization of the local infrastructure is necessary to make it possible to disseminate technical information among dairy farmers. The development of a system of credit is necessary to facilitate the acquisition of feed ingredients and farm equipment. The establishment of AI service units at the regional level will encourage the stockbreeders to manage and prepare their animals for insemination and will also avoid the delays that occur in serving the animals. The organization of training courses for AI technicians will help to increase the success rate.

The system of management of crossbreds needs to be improved, with better housing and quality of fodder. The installation of a network for marketing of products is necessary to facilitate the flow of economic benefits. The participation of women should also be encouraged in the activities related to milk production.

AI has succeeded in raising the levels of production of meat and milk by generating F1 crossbreds from the local breeds of cows, but the success rate in terms of calving are low. Although the main costs for AI are subsidized by the Senegalese State, benefits reported in monetary terms are low. Future studies should be aimed at reducing the costs and increasing the benefits, in order to encourage dairy producers to adopt AI on a larger scale.

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PREGNANCY DIAGNOSIS IN DAIRY COWS USING MILK PROGESTERONE DETERMINATION BY RADIOIMMUNOASSAY

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Abstract

Milk samples were analysed for progesterone by radioimmunoassay (RIA) as an indicator of the pregnancy status in 582 Holstein cows varying in age between 30 and 65 months, parities between 2 and 5 and body weights between 380 and 650 kg. Detection of oestrus was based on overt signs and breeding was done exclusively by artificial insemination (AI). The number of days post-AI when milk was sampled had an effect on pregnancy diagnosis by milk progesterone. Milk samples between day 22 and 26 post-AI provided an accurate diagnosis of pregnancy, with day 23 being most accurate. The milk progesterone test was 98.3% accurate for non-pregnancy and 90.4% accurate for pregnancy. A double milk sampling routine (days 21 and 22 post-AI) resulted in 1.7% greater accuracy for pregnancy diagnosis. The reference value of 3 nmol/L and above for milk progesterone for positive pregnancy diagnosis was acceptable. Accuracy, repeatability and practicability of the pregnancy diagnosis were the most important factors that determined the feasibility of the test. The determination of milk progesterone by RIA was found to be consistent and acceptable when combined with subsequent rectal palpation. However, factors such as oestrus detection and nutritional level may affect the accuracy. Milk progesterone determinations definitely have a role to play in the pregnancy diagnosis of dairy herds.

1. INTRODUCTION

Progesterone concentrations in blood and milk of cows are closely related to the growth and secretory function of the corpus luteum (CL) during the normal oestrous cycle and pregnancy. After ovulation and formation of a CL, high levels of milk progesterone are reached on approximately day 14 (day 0=oestrus) of the cycle. If the cow becomes pregnant the active CL is maintained and progesterone secretion persists [1]. In the non-pregnant cow, however, the milk progesterone level rapidly declines from day 17 of the cycle [2]. The difference recorded in milk progesterone concentration between pregnant and non-pregnant cows is greatest between days 20 to 24 following AI. This difference in milk progesterone levels forms the basis of the test for early non-pregnancy diagnosis in cattle [1]. Pregnancy diagnosis is thus dependent on the presence or absence of a functional CL in the ovary, 20 to 24 days after insemination. Discriminatory levels of milk progesterone must be set to enable the differentiation between an active CL, a regressing CL or the absence of a CL [3].

In this study the milk progesterone test for pregnancy diagnosis was evaluated in dairy cattle to determine: (a) the accuracy of the test, and (b) the discriminatory limit of milk progesterone indicating the presence of an active CL and possible pregnancy.

2. MATERIALS AND METHODS

2.1. Farms, animals and records

The survey was conducted on 4 dairy farms in Gauteng and Mpumalanga, South Africa, each with more than 150 cows in milk. Five hundred eighty-two (582) Holstein dairy cows, varying in age from 30 to 65 months, were utilized in this study. The parity of the cows varied between 2 and 5, body condition score (BCS) at calving ranged between 2 and 4.5 (on a 5-point scale) [4] and body weight (BW) ranged between 380 kg to 650 kg. Post partum uterine involution, ovarian activity and fertility parameters were monitored in all cows.

Feeding in the herds was based on improved pastures with supplementation of maize silage (20–25 kg/cow/day), hay *ad libitum* and concentrates (10–13 kg/cow/day) with 17–19% protein content and a relative energy value of 12 MJME/kg DM, fed in the form of a mixed diet throughout the year. Machine milking was carried out twice daily (06:00 and 18:00 hr) on three farms and three times per day (06:00, 14:00 and 22:00 hr) on the fourth farm. The oestrous detection routine used was based on overt signs (cows standing to be mounted) and these observations were carried out twice a day at the time when cows were moved to the milking parlour (06:00 and 18:00). The time devoted to observation of the cows in oestrus was approximately one hour in the morning and again an hour in the afternoon. Artificial Insemination (AI) was done exclusively on all farms, 12–14 hours after standing oestrus, with no clean up bulls being used in the breeding system.

The data collected included calving date, parity (1=first-calf heifers and 2=mature cows), ease of calving (1=normal, 2=simple assistance and 3=major assistance), breeding dates, pregnancy diagnosis, monthly milk production, BW and BCS at calving and at each breeding, and milk progesterone levels to evaluate the efficiency of oestrous detection.

Information was collected in a database designed especially for this purpose. The BCS was assessed on a scale of 1 to 5 [4], but for the statistical analysis cows were grouped as low BCS (≤2) and moderate BCS (>2). The milk production of each cow was recorded daily and reproductive events were recorded until the cow was certified pregnant or culled. Pregnancy diagnosis was performed by rectal palpation 60 days after AI.

2.2. Milk sampling and progesterone assay

Milk samples for pregnancy diagnosis by progesterone measurement were obtained from the 4 dairy herds. In 3 of the herds, a single sample was taken between days 20 and 24 after AI. In the fourth herd, two milk samples were taken on day 21 and day 22 after AI. Milk samples were collected in 20 mL vials containing sodium azide (Merck KgaA, Damstadt, Germany) as a preservative and stored at 4°C on the farm for later transport to the radioimmunoassay (RIA) laboratory. There, milk samples were centrifuged and the fat free fraction was stored at -20°C until analysed for progesterone concentration with the aid of a solid phase RIA method. The intra-assay coefficient of variation (CV) was 8.2% for samples with progesterone values below 1 nmol/L and 9.8% for samples with a value above 1 nmol/L. The inter-assay CV was 11.7 and 4.5% for samples with progesterone values below or above 1 nmol/L, respectively.

Three aspects were analysed regarding the use of milk progesterone for pregnancy diagnosis in cows. The first was to set the discriminatory progesterone limit for determining the presence or absence of a CL. This was done by discriminant analysis (a statistical package available on the Statistical Packages for Social Sciences) using the milk progesterone concentration at day 21 in cows subsequently confirmed pregnant. The results from 582 milk samples (i.e. 376 pregnant diagnoses and 206 non-pregnant diagnoses) formed the basis of this analysis. Using the milk progesterone level of the sample and the outcome of insemination, the discriminant function was calculated. From the discriminant function, several milk progesterone concentrations were evaluated to calculate at which level the frequency of false positive and false negative diagnoses was minimised. Logit analysis was then performed to test the findings of discriminant analysis.

A second analysis was performed to determine whether the day of sampling affected the accuracy of classification for pregnancy in cows. This analysis utilized the data obtained from complete milk progesterone profiles of 76 cows over 136 inseminations. The third feature tested was to establish whether two consecutive milk samples, taken on day 21 and day 22 after AI, gave more accurate results than one sample taken between days 20 to 24 after AI. This analysis was based on 196 pregnancy diagnoses done using a single sample and 386 pregnancy diagnoses done using the double sampling method.

2.3. Statistical analysis

The statistical analysis of the intervals from calving to first service and from calving to conception was carried out according to the following general linear model (SAS 1990 SAS Procedures Guide (Version 6, 3rd Ed) SAS Inst. Inc. Cary, NY, USA):

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

Where INT is the interval to first service or to conception; b is the *j*th effect of farm (A, B, C, D and E); c is the *k*th effect of parity (1 and 2); d is the *l*th effect of ease of calving (1, 2, 3 and 4); e is the *m*th effect of BW (<500 and >500kg) f is the *n*th effect of BCS at calving (1 and 2); g is the *o*th effect of month of calving (1–7); h is the *p*th effect of month of breeding (5–12); I is the *q*th effect of BW at service (<500 and >500 kg); j is the *r*th effect of BCS at service (1 and 2); k is the *s*th effect of milk production at service (5, 10, 15, 20, 25 and 30 L) and $\varepsilon_{jklmnopqrs}$ is the error.

Interactions between variables were tested and mean comparison was done using the LSD method. To analyse conception rate at first service and overall pregnancy rate the CATMOD procedure was utilized.

3. RESULTS

3.1 Discriminant analysis

The discriminant analysis (Table I) demonstrated no difference in the overall classification at a level of 3.0 nmol/L or 3.5 nmol/L milk progesterone. The Pearson correlation was higher at the 3.0 nmol/L milk progesterone levels.

TABLE I. ACCURACY OF PREGNANCY DIAGNOSIS IN COWS AT DIFFERENT MILK PROGESTERONE LEVELS (DISCRIMINANT ANALYSIS)

Milk progesterone concentration (nmol/L)	Correct diagnosis of cows (%)			Pearson Correlation
	Non-pregnant	Pregnant	Overall	
2.0	92.0	99.5	95.8	91.1
2.5	93.2	98.9	96.1	93.2
3.0	97.1	98.9	98.0	96.2
3.5	98.1	97.9	98.0	95.5
4.0	98.1	94.7	96.4	91.3

The logit analysis (Table II) provided slightly more sensitive results. With this analysis, a level of 3 nmol/L proved to be the most accurate in predicting pregnancy, although only marginally so. When combining the results of the discriminant and logit analysis, a threshold for milk progesterone of 3 nmol/L was established to distinguish between pregnant and non-pregnant cows.

TABLE II. ACCURACY OF PREGNANCY DIAGNOSIS IN COWS WITH DIFFERENT MILK PROGESTERONE CONCENTRATIONS (LOGIT ANALYSIS)

Milk progesterone concentration (nmol/L)	Correct diagnosis of cows (%)			Pearson Correlation
	Non-pregnant	Pregnant	Overall	
2.0	92.0	99.5	95.8	91.1
2.5	96.0	98.9	97.5	93.2
3.0	97.1	98.4	97.9	95.5
3.5	97.1	97.9	97.6	94.8
4.0	98.1	94.7	95.9	91.3

3.2. Day of sampling

The number of days post insemination when a milk sample was taken had a definite affect on the accuracy of pregnancy classification in pregnant and non-pregnant cows (Table III).

Milk samples taken between days 22 to 26 post-AI provided accurate diagnosis of pregnancy, with the day 23 sample proving the most accurate for classification. Outside this time frame, the percentage of cows incorrectly classified as pregnant was high. This result was expected for samples taken before day 18 or after day 27, but not for milk samples collected between 19 and 21 days following AI.

3.3. Number of milk samples used in pregnancy test

Before the investigation it was assumed that two milk samples, taken on days 21 and 22 post-AI, would provide a more accurate diagnosis of pregnancy than a single sample taken between 20 and 24 days. The extent to which the two procedures differed was of interest and Table IV presents a summary of the results with regard to the number of milk samples used.

TABLE III. EFFECT OF DAY OF MILK SAMPLING ON THE ACCURACY OF PREGNANT DIAGNOSIS

Day on which sample was taken (day 0 = day of AI)	Cows incorrectly diagnosed (%)
17	23.0
18	18.0
19	7.8
20	7.6
21	5.0
22	2.1
23	1.4
24	1.6
25	2.1
26	2.0
27	3.1
28	4.8

TABLE IV. EFFECT OF NUMBER OF MILK SAMPLES USED ON THE ACCURACY OF PREGNANCY TEST IN COWS

Number of samples	Sample induced error rates (%)	
	Incorrect pregnant diagnoses	Incorrect non-pregnant diagnoses
1	6.5	3.4
2	4.8	3.8

The results confirm that a double milk sampling routine was more accurate in pregnancy diagnosis. For non-pregnant cows, diagnostic error rates were virtually identical. Overall, the analysis of two milk samples improved the accuracy of pregnancy diagnosis by 1.7%, a difference much smaller than expected.

3.4. Accuracy of the milk pregnancy test

Using a milk progesterone concentration of 3 nmol/L as the reference level, the reliability of pregnancy diagnosis in dairy cows was determined.

The progesterone test for diagnosing non-pregnancy in cows was 98.3% accurate and the diagnosis for pregnancy was 90.4% accurate. Overall, an accuracy of 94.4% for diagnosis of pregnancy was achieved using the milk progesterone assay on 21–24 days post-AI.

4. DISCUSSION

The reference level of milk progesterone of 3 nmol/L established for pregnancy diagnosis in cows in the present study was similar to that reported by other researchers [5–7]. In some studies, upper and lower discriminatory reference levels were introduced, with cows recording milk progesterone levels within these limits being classified as ‘doubtful’ [8, 9]. However, the single discriminatory progesterone reference level set in the current progesterone assay was shown to be very accurate and repeatable. There was thus no need to set upper and lower limits for confirmation of pregnancy. In addition, only 6% of the milk samples analysed had milk progesterone concentrations between 2 and 4 nmol/L. These few samples were classified using the single discriminatory reference limit. The close relationship between the discriminant levels and logit analysis of the data tended to indicate that the discriminant function could possibly be used to set cut off limits in the diagnosis.

The results of this study emphasized the importance of the day of milk sampling on the accuracy of pregnancy diagnosis. Usually, a milk sample is usually taken 20 to 25 days after insemination as this range tends to cover the known variation in oestrous cycle length. According to the present results, milk samples taken during the latter half of this period (i.e. days 22 to 25) are more accurate for confirming pregnancy than those taken earlier (i.e. days 20 and 21). In a previous study, the maximum accuracy was obtained from a milk sample on day 23 following AI [10], but other studies claimed maximum accuracy to be achieved 21 to 23 days after AI [6, 11]. However, the day 23 milk sampling procedure has the advantage in that farmers are able to exclude from sampling any cows that have demonstrated oestrus before this time.

The double milk sampling routine on 21 and 22 days post-AI has the advantage over a single milk sample in circumstances when milk progesterone concentration is still decreasing to a basal level. This occurs when the oestrous cycle length is slightly longer, or milk sampling is done too early. The first milk sample may have a progesterone level higher than the discriminatory reference limit, leading to an incorrect pregnancy diagnosis. A second sample, on the other hand, may demonstrate that the milk progesterone concentration is still decreasing and thus a correct diagnosis can be made. Although slight improvement in the accuracy of pregnancy diagnosis was observed when using two samples rather than one in the current study, the difference was not sufficient to warrant additional expense of taking of a second sample. In practice, a double milk sampling routine would imply more labour, time and financial implications. Thus, it would seem that the single sampling technique would be a more commercially viable option.

Accuracy and repeatability of pregnancy diagnosis are the most important factors that determine the feasibility of the milk progesterone pregnancy test. The RIA for determining the milk progesterone in this study was found to be highly consistent, thus the misdiagnosis of certain cows may have been the result of other factors. A number of on-farm practices like method of oestrus detection and nutritional management can affect the accuracy of the test.

Milk progesterone concentrations are highly correlated with the milk fat concentration. Thus the quality of the milk sample drawn from the cow and subsequent processing to remove the fat before conducting the progesterone assay must be consistent with the milk used in the standard curve of the assay. Any deviation from this could lead to erroneous results and complicate the interpretation of the data [9].

If an insemination is performed during the luteal phase of the oestrous cycle, the milk test sample will be drawn during the corresponding luteal phase of the subsequent cycle, thus giving a false positive result. The record keeping on herd management is thus important to ensure that inseminations are appropriately performed, and that the test samples are taken from the right cows on the right days. Studies have shown the accuracy of the milk progesterone tests to vary between farms, due to different farm management practices including the accuracy of record keeping [12].

Milk progesterone concentration during the oestrous cycles in dairy cows follows the same basic pattern, but irregularities and deviations are not uncommon. These deviations may then contribute to test inaccuracies, and must not be ignored. For example, ovulation may have occurred at the normal time, but the resulting CL may have a life span shorter or longer than normal. The subsequent milk progesterone concentration at the time of testing may thus be higher than the discriminatory reference limit, leading to a false positive diagnosis [13]. Other irregularities that may occur in milk progesterone concentration result from reproductive disorders such as luteal cysts and persistent CLs.

Late embryonic losses appear to be the main cause of incorrect positive pregnancy predictions [14]. Such losses have been well documented in lactating cows and may range from 10 to 25% in well managed dairy herds. The extent of these embryonic losses could explain the false positive pregnancy diagnoses described in previous studies [15–17]. It may not be right to include late embryo loss in the false positive diagnosis category, as the cow could actually have been pregnant on the day of sampling. If this were the case, the accuracy of pregnancy diagnosis of the cows in this study would increase to 97.4%, leaving a 2.6% error due to herd management or oestrous cycle abnormalities. However, in the eyes of the farmer the RIA milk progesterone test gave an acceptable result and embryo mortalities must be included when determining the precision of the milk pregnancy assay.

The overall diagnostic accuracy of 90.4% for pregnant and 98.3% for non-pregnant cows in the present study are similar to results obtained by other researchers, with accuracy rates between 81–91% [18], 85.1–98.8% [19] and 94.7–95.2% [20] being reported. Under commercial dairy conditions the accuracy of the positive pregnancy test will probably be lower than that recorded under research conditions, due to lower resources for managing the sampling procedures and assay facilities. A high occurrence of inaccuracy in pregnancy diagnosis would be unacceptable to any dairy farmer. This has prompted the opinion that the only information obtained from a milk progesterone pregnancy test, which can completely substitute the clinical pregnancy examination, is when progesterone levels are below the discriminatory limit [18]. Thus it would seem the principle objective of the milk pregnancy test is to detect cows that are not pregnant, which is satisfying the aim of pregnancy diagnosis in dairy cattle.

In conclusion, the pregnancy test was accurate, provided record keeping was efficient and up to date. The 90.4% accuracy for positive pregnancy diagnosis was acceptable when factors affecting this test are taken into consideration. This success rate would have been lower if record-keeping on the farms had been poor. On numerous occasions when a false positive result was recorded, a check of the records indicated an incorrect sampling time. Such samples were consequently excluded from the analyses, and support the contention that the value of the test is in the detection of non-pregnant cows, rather than pregnant cows.

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THE EFFECT OF FEEDING DIFFERENT LEVELS OF PROTEIN AND ENERGY ON THE REPRODUCTIVE PERFORMANCE OF SUDANESE NUBIAN GOATS

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Abstract

Forty-four Sudanese Nubian goats were divided into four groups. Each group was offered a ration containing energy (E) and protein (P) at levels that were either high (H) or low (L). The groups were designated as HEHP, LELP, HELP and LEHP. The animals were synchronized using an intra-vaginal slow release progesterone device for 14 days, followed by injection of PMSG at withdrawal. Animals that were detected in heat by visual observation were mated by bucks. The reproductive parameters were investigated throughout pregnancy, kidding and up to 5 months post partum. Samples of milk and blood (for serum and plasma) were collected at weekly intervals and progesterone was measured by radioimmunoassay. The HEHP group showed the best reproductive performance with 63.6% conception rate (CR), 85.7% maintenance of pregnancy and 100% kid survival rate. This group had the shortest interval to post-partum progesterone elevation, indicating early resumption of ovarian cyclicity. The LELP group also had high CR (63.6%), but had the shortest gestation period, high percentage of embryonic and foetal losses, low birth weight of kids and 100% kid mortality rate. This group had the longest interval to post-partum progesterone elevation (83–159 days). The CR in the HELP group (60%) was comparable to that of the LEHP group (54.5 %). Both these groups had intermediate levels of embryonic losses and kid mortality. Monitoring the progesterone profiles of individual does together with recording of clinical observations enabled an accurate diagnosis of conception, the course of pregnancy and the time of initiation of post-partum ovarian activity. It is concluded that nutritional status influences the maintenance of pregnancy, kidding rate, weight of kids at birth and kid survival in Sudanese dairy Nubian goats.

1. INTRODUCTION

Sudan has a wealth of livestock resources. The contribution of animal production to GDP was estimated at 20% in 1997, which is equivalent to 42% of the contribution of the agricultural sector. Livestock numbers were estimated at about 103 million in 1994/1995 of which goats constitutes 37 million [1]. Goats make an important contribution to national food security by providing meat and milk at very low cost. Moreover, during the year 2000 they contributed to the national economy through exportation of 315 tons of meat and 66 000 live goats [2].

Several breeds of goats are known to exist in Sudan. However; the Nubian breed is the most important and widespread breed, which is raised mainly for milk production and to a lesser extent for meat. It is known that improved feeding practices and good housing facilities result in optimum growth, high milk yield and better reproductive performance in goats. Furthermore, poor nutrition was identified as the main cause of poor reproductive performance of dairy cattle in the Sudan [3] and this also may be true in goats. Therefore, introduction of improved feeding practices based on strategic balanced rations could enhance both production and reproductive performance of dairy goats. Among the major nutrients, water is the most important, followed by energy and protein. Without adequate energy, utilization of all nutrients is impaired. Energy is required for a variety of functions like maintenance, reproduction and lactation; however, the utilization of feed energy is governed largely by the type of ration fed. Protein is needed for the normal metabolic functions of building and repair of tissues as well as the demands for production and reproduction.

In spite of the fact that Sudan has a large population of goats, little effort has been made to improve their reproductive performance. The main objectives of this study were to (a) determine the effects of different levels of energy and protein in the diet of Nubian goats on their productivity and reproductive efficiency, and (b) to study their hormonal profiles in order to accurately assess reproductive performance.

2. MATERIALS AND METHODS

2.1. Area of study

This study was carried out at the Small Ruminant Unit of the Animal Production Centre, located at Helat Kuku, Khartoum North, between latitude 150°36’ North and longitude 320°33’ East at an altitude of 380 m above sea level. The period of study lasted from May 2003 to July 2004.

The small ruminant unit had a flock of 117 pure adult Nubian does and five bucks. They were originally brought from the Abu Hamad area of the Northern State in 1992. They were all kept in a shaded area of about 20x20 m with a bamboo roof and a free fenced area of 20x30 m.

2.2. Experimental animals

A total of 44 healthy adult mature female Nubian dairy goats and 3 adult mature bucks were selected from the above flock. The bucks were housed separately in pens, each of 20 m^2. They were provided with a maintenance ration and water *ad lib*. Three weeks prior to breeding, they were offered a supplementary ration consisting of sorghum, groundnut cake and wheat bran (50:25:25). The does were housed in pairs in separate cages, 1.5x2.5 m each, and were randomly divided into four groups, so that their average initial mean body weight was approximately the same in all groups.

The animals were kept for 4 weeks in cages to adapt and to be clinically examined for freedom from diseases, especially brucellosis, reproductive disorders and other abnormalities.

2.3. Experimental Ration

The does were fed four experimental diets as follows: (a) high energy, high protein (HEHP); (b) low energy, low protein (LELP); (c) high energy, low protein (HELP); and (d) low energy, high protein (LEHP).

The rations were basically calculated using the production ration for lactating does as given in Nutrient Requirements of Goats [4]. The HE and HP diets comprised 3.12 Mcal /kg dry matter (DM) and a total protein of at least 13.1%, respectively. The LE and LP diets (maintenance ration) had 2.22 Mcal/kg DM and at least 7.3% protein, respectively. The rations were formulated using the computer program Feedwin (Win 95) for feed formulation (ICP Livestock, Barneveld, 1997-1998). The ingredients for all diets were ground sorghum stalks, sorghum, molasses, groundnut cake and wheat bran. The chemical composition of ingredients was estimated using the values obtained from the

Nutrient Composition of Sudanese Animal Feeds [5] and then confirmed by laboratory analyses .The formulation of the various rations is shown in Table I, while Table II gives their proximate analysis.

TABLE I. PERCENTAGE COMPOSITION OF INGREDIENTS IN THE FOUR EXPERIMENTAL DIETS

Ingredient	Diet[a]			
	HEHP	LELP	HELP	LEHP
Sorghum stalk	20	60	40	51
Sorghum	40	10	30	0
Molasses	10	10	19	40
Groundnut cake	5	0	0	0
Wheat bran	24	19	10	5
Urea	0	0	0	3
Salt	1	1	1	1
Total %	100	100	100	100

[a]H = High, L = Low, E = Energy, P = Protein

TABLE II. PROXIMATE FEED ANALYSIS FOR THE DIFFERENT DIETS

Diet[a]	Dry Matter (%)	Ash (%)	Crude Protein (%)	Ether Extract (%)	Crude Fibre (%)	Nitrogen Free Extract (%)	Metabolizable Energy (mj/kg DM)
HEHP	93.00	7.30	16.07	3.20	10.2	56.23	12.14
LELP	93.80	9.30	7.36	1.40	22.8	52.94	10.29
HELP	93.00	9.00	7.36	1.60	14.8	60.24	11.15
LEHP	90.60	10.70	15.40	1.80	17.2	45.50	10.29

[a]H = High, L = Low, E = Energy, P = Protein

Each group of animals was fed its specified ration. Daily records were kept for the intake by subtracting the daily refusal from the amount offered.

2.4. Oestrous synchronization

Ten weeks after starting the feeding regime with the four diets, the animals in each group were synchronized on the same day, with an interval of 4 days between the groups. Synchronization was done by insertion of a Controlled Intravaginal Drug Release (CIDR) device that contained 0.3 g slow release progesterone (Inter Ag, Netherlands). The CIDR was removed after 14 days [6]. At the time of CIDR withdrawal each goat, according to its live weight, received an intramuscular injection of 400–500 IU of Pregnant Mare Serum Gonadotrophin (PMSG, Intervet, UK).

2.5. Mating

Heat was detected by visual observations after the removal of CIDR. Animals were considered to be in heat if they showed one or more signs such as tail wagging, a swollen and red vulva with mucus discharge, mounting on each other, frequent urination, increased activity rate, nervousness, and reduced appetite and milk production. Such animals were hand mated by presenting them one-by-one to the buck, and ensuring that breeding occurred at least twice per doe.

2.6. Sampling protocol and progesterone measurement

To obtain an accurate profile of changes in progesterone in each doe, the progesterone concentration in samples of milk, blood serum and plasma were determined by radioimmunoassay (RIA) technique. The reason for three types of samples was to compare the different levels of progesterone in these media in Sudanese Nubian goats.

2.6.1. Milk

Milk samples (10 mL) were collected into Mac-Cartney bottles, each of which contained one tablet of sodium azide (100 mg) as a preservative, at weekly intervals throughout the study. They were centrifuged at 3000 rpm for 15 minutes to remove the fat and the skimmed milk was stored in sealed plastic containers at -20°C until analysed. The concentration of progesterone was measured using a solid-phase radioimmunoassay (RIA) method, using kits supplied by the joint FAO/IAEA division ('Self-Coating' Milk Progesterone RIA, Bench Protocol Version 3.1, 1999). Inter-assay coefficient of variation (CV) for two sets of internal laboratory quality control values were 12.9% and 12.6%, respectively. Progesterone concentrations greater than 1 nmol/L were considered as indicative of the presence of a corpus luteum (CL) in the ovaries.

2.6.2. Serum and Plasma

Weekly blood samples (3–5 mL) were collected into vacutainer tubes with and without heparin from each goat throughout the study. Heparinized blood samples were immediately centrifuged at 3000 rpm for 15 minutes and the plasma was removed and stored at -20°C until analysed. Blood samples without heparin were first allowed to clot and then centrifuged to separate the serum, which was also stored at -20°C until analysed.

Serum and plasma progesterone concentrations were measured using RK-460 M progesterone RIA kits (supplied by the Institute of Isotopes Co. Ltd., Budapest). The technique was basically a competition method using antibody-coated magnetic particles. The sample and reagents were mixed in one tube and the bound fraction was separated using either the magnetic separator or centrifugation method, according to the manufacturer's protocol. Six serum standards ranging from 6–120 nmol/L were used together with a quality control (QC) sample (expected range of 24.7–37.2 nmol/L). The mean value obtained for the QC sample was 33.31+2.36 nmol/L, with an inter-assay CV of 7.09%.

The progesterone profile of each doe was examined in relation to the mating date. Conception was determined to have occurred when three successive samples had progesterone concentration above 3.0 nmol/L. This was later confirmed by the date of delivery of kids.

2.7. Statistical analysis

The data obtained were subjected to statistical analysis using the computer program SPSS 10.0 for Windows. Differences in mean values between groups were tested by ANOVA or Chi- square test and the level was considered significant when $P < 0.05$.

3. RESULTS

In each of the four groups, 10 out of the 11 animals showed heat signs 48–72 hours after the injection of PMSG. All does except one from the HELP group survived until the end of the study. Table III summarizes the data on conception rate (CR), kidding, embryonic and foetal losses and post-partum non-cyclicity in the four groups.

TABLE III. THE NUMBERS AND PERCENTAGES OF ANIMALS THAT CONCEIVED, KIDDED, HAD EMBRYONIC OR FOETAL LOSSES, AND WERE NON-CYCLIC POST-PARTUM

Group[a]	No. of animals	Conceived	Kidded	Embryonic and foetal loss	Post-partum non-cyclic
HEHP	11	7/11 (63.6%)	6/7 (850%)	1/7 (14%)	0 (0%)
LELP	11	7/11 (63.6%)	4/7 (57.1%)	3/7 (42.8%)	2/7 (28.6%)
HELP	10	6/10 (60%)	3/6 (50%)	3/6 (50%)	3/6 (50%)
LEHP	11	6/11 (54.5%)	4/6 (66.7%)	2/6 (33.3%)	1/6 (16.7%)

[a]H= High; L= Low; E= Energy; P = Protein

The gestation period and post-partum interval to the first elevation of progesterone rise are shown in Table IV. The data on mean body weight of kidded does, prolificacy, kid body weight and kid survival rate are given in Table V.

Figure 1 shows the progesterone profiles obtained for one doe using milk, serum and plasma samples. The profile for milk progesterone indicated that the animal had a somewhat irregular cyclic pattern, followed by pregnancy around 30 weeks after commencement of sampling. This was also reflected in the profiles for serum and plasma, with some variability in the magnitude of peak concentrations.

TABLE IV. GESTATION PERIOD AND INTERVAL FROM KIDDING TO THE FIRST ELEVATION OF PROGESTERONE (MEAN ± SD; RANGE IN PARENTHESIS)

Group[a]	Gestation period (days)	Interval to progesterone elevation (days)
HEHP	157.0 ± 5.2[b] (151–163)	44.6 ± 18.1 (21–67)
LELP	141.3 ± 2.2[c] (139–144)	121.0 ± 53.7 (83–159)
HELP	144.3 ± 4.0[bc] (140–148)	83.5 ± 81.3 (26–141)
LEHP	153.3 ± 14.7[bc] (132–163)	47.3±42.2 (21–96)
P value	0.050*	0.210

[a]H= High; L= Low; E= Energy; P = Protein. *Differences between means denoted by dissimilar superscripts are significant at 0.05 (ANOVA test)

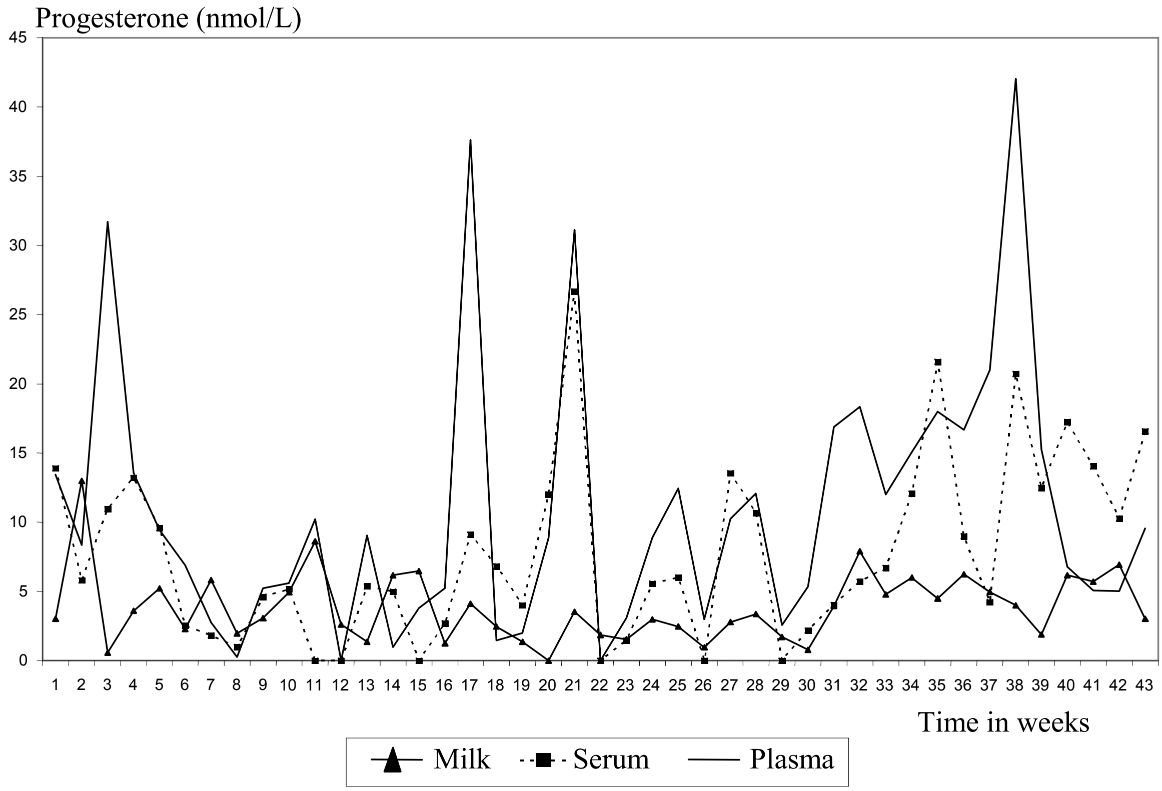

FIG. 1. Milk, serum and plasma progesterone profiles in one doe, showing an irregular cyclic pattern followed by pregnancy at around 30 weeks from commencement of sampling

TABLE V. BODY WEIGHT OF DOES AT KIDDING, PROLIFICACY, KID WEIGHT AND KID SURVIVAL RATE (MEAN ± SD; NUMBER OF OBSERVATIONS IN PARENTHESIS)

Group[a]	Body weight at kidding (kg)	Prolificacy (Kids/doe)	Kid weight (kg)	Kid survival Rate (%)
HEHP	22.8 ± 1.8 (5)	1.8 ± 0.8 (5)	1.6 ± 0.3 (9)	100
LELP	23.2 ± 2.0 (3)	2.3 ± 0.9 (4)	1.4 ± 0.1 (9)	0
HELP	20.8 ± 1.1 (2)	1.5 ± 0.7 (2)	1.3 ± 0.2 (3)	33.3
LEHP	22.5 ± 2.8 (2)	1.0 ± 0.0 (2)	1.4 ± 0.0 (2)	0

Figure 2 depicts a representative serum progesterone profile of a doe that became pregnant, kidded and resumed cyclicity early during the port-partum period. Figure 3 shows the serum progesterone profile of a doe that became pregnant and underwent foetal death.

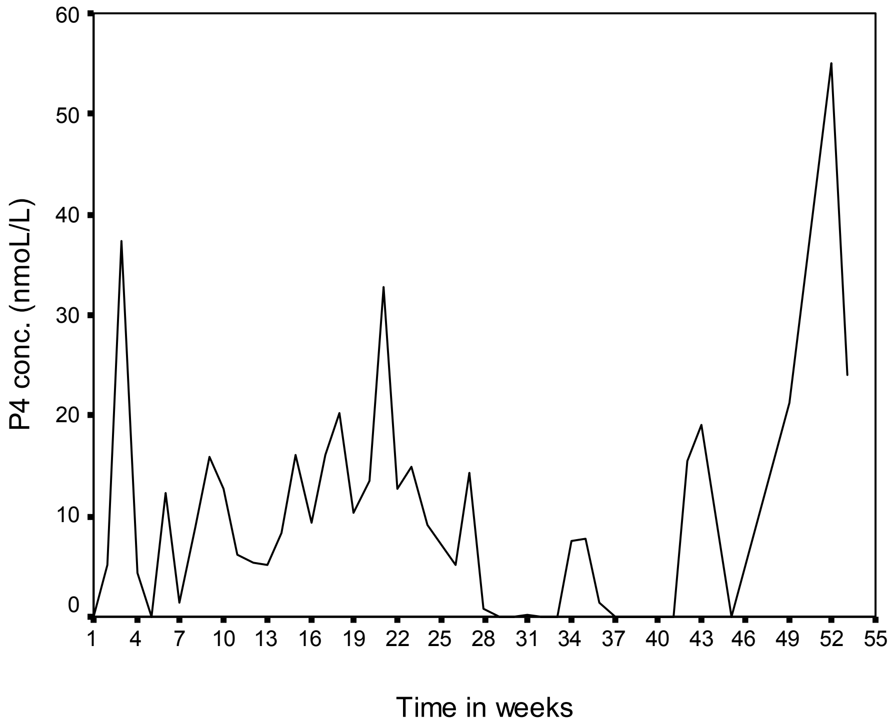

FIG. 2. Serum progesterone (P4) profile in a doe, showing pregnancy and kidding followed by early resumption of cyclicity

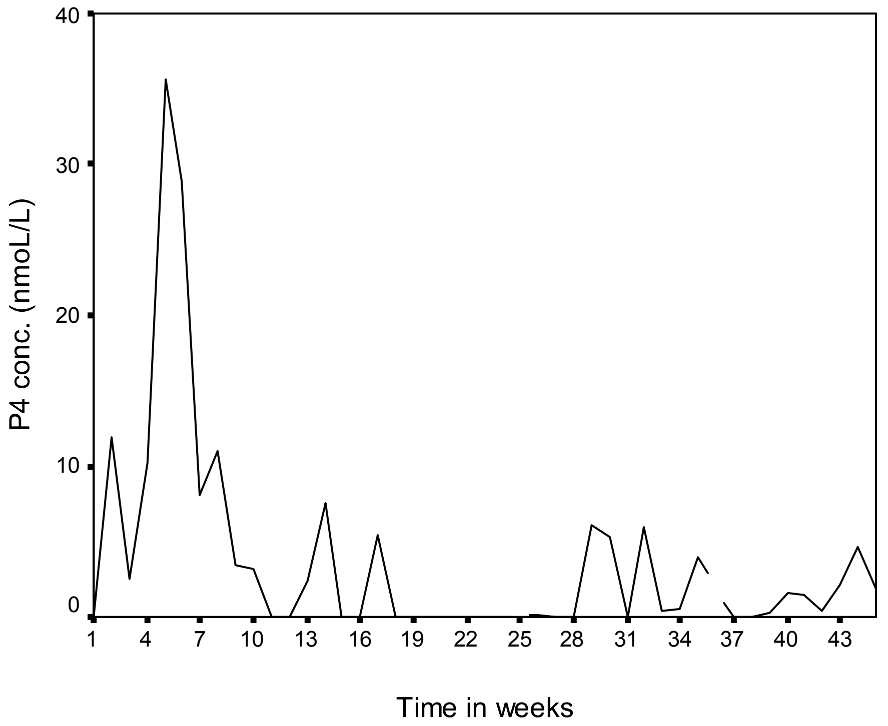

FIG. 3. Serum progesterone (P4) profile in a doe, showing conception followed by foetal death at around 11 weeks of pregnancy

4. DISCUSSION

The availability of food nutrients is considered to be the ultimate regulator of the reproductive functions. Severe under nutrition in goats can lead to cessation of all reproductive activity regardless of other factors [7]. While liberal feeding enhances early sexual maturity, underfeeding causes temporary sterility and excessive thinness of does results in low birth weight and weak kids [8]. However, there are few detailed investigations on the effects of different feeding regimes on reproduction in goats.

In the present study the proportion of goats that showed heat signs at 48–72 hours after injection of PMSG was higher in the HEHP group (100%) than in the other three groups (81–90%). Thus, pre-mating supplementation of crude protein and energy appeared to enhance the incidence of oestrus after synchronization. This is in agreement with findings in Mashona goats [9] where dietary energy restriction during pre-mating was found to decrease the proportion of does showing onset of oestrus. On the contrary, studies on Saanen and Toggenburg goats [10] reported that incidence of oestrus was not affected by energy deprivation, although the interval to onset was longer.

The CR in the four groups ranged from 54–63%, with no significant difference between the groups. This finding was similar to those in Damascus [11] and Thai goats [12], where different regimes of concentrate supplementation did not increase the CR. In other studies, however, levels of low, medium and high energy resulted in lower CR in the low energy group than in the other two [9, 13] and the feeding of pods of a tree, which were high in protein and energy, resulted in higher CR than in control goats [14].

The mean gestation length in the LELP group was significantly lower than that in the HEHP group ($P<0.05$), while there was no significant difference between the other two groups. Previous studies [15, 16] have reported similar gestation lengths in control and supplemented goats, while others [17] have found no significant effects on gestation length of Baraki ewes or dairy goats. It has been speculated [18] that low protein intake results in lower foetal growth rate and consequently prolonged gestation length. Breed is also known to influence gestation length, with Alpine goats having a mean duration of 151.6 days and Nubian goats a mean duration of 149.2 days [19]. Goats in Fiji and Western Samoa have been reported have gestation lengths ranging from 140–158 days [20].

The mean interval from kidding to the first elevation of progesterone was 44.4, 47.33, 83.5 and 121 days for the HEHP, LEHP, HELP and LELP groups, respectively. Therefore, it appears that ovarian activity is affected more strongly by a diet low in protein than one low in energy. Our finding that the mean body weights of does that kidded in the different groups were not significantly different ($P >0.05$) is similar to a previous study [21] where the body weight at kidding did not affect resumption of ovarian activity or oestrus. On the other hand, the mean birth weight of kids was higher in the HEHP group than in the other three groups. Their survival rate was also highest in the HEHP group. Supplementation with energy [18] and protein [22] has been shown to improve kid birth weight [15], although some other studies have failed to show similar results [12].

The number of kids per doe (prolificacy) is known to be increased by high energy diets [24] and decreased by restriction of dietary energy [9]. In another study, however, no significant increase occurred in kidding rate or multiple births when goats were fed supplementary feeds for 15 days before and 45 days after mating [12]. In the present study prolificacy was highest in the LELP group, which is contrary to previous studies, but the numbers of animals per group was too small to draw definite conclusions on this parameter.

The effect of nutrition on embryo survival has been linked with peripheral progesterone concentrations. Overfeeding during early pregnancy resulted in reduced progesterone levels and elevated embryo loss in sheep [23]. Also, progesterone levels were higher in does on a maintenance ration than in those provided supplements [25–27]. On the contrary, higher plasma progesterone was reported in pregnant ewes fed a high level of nutrition than in those fed on a low level [28], but pre-mating nutritional treatments had no effect on progesterone levels [29]. In cattle, it has been reported that dietary protein can alter the secretion of progesterone and LH [30, 31].

This study has shown that a complex relationship exists between the levels of crude protein and energy in the diet and reproductive functions in dairy goats. Deficiency in protein appears to have a more marked effect than deficiency in energy. Monitoring the concentration of progesterone in milk, serum and plasma showed that the profiles were comparable in each animal, and either could be used for determining the reproductive status. It is concluded that nutritional status influences the maintenance of pregnancy, kidding rate, weight of kids at birth and kid survival in Sudanese dairy Nubian goats.

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USE OF PROGESTERONE MEASUREMENT TO MONITOR ARTIFICIAL INSEMINATION, REPRODUCTIVE FUNCTIONS AND PREGNANCY IN TUNISIAN CATTLE

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Abstract

A study was conducted on 924 dairy cows subjected to artificial insemination (AI) in four Tunisian farms. Three milk samples were collected from each cow, at the time of AI (day 0), 12 days after AI and between 22–24 days after AI. A total of 2830 milk samples were collected and progesterone was measured by radioimmunoassay (RIA). Results from samples collected on day 0 (n=1034) revealed that 10.1% of cows was inseminated at improper times, when progesterone level was greater than 1 nmol/L. Results from samples collected 12 days after AI (n=948) allowed distinguishing between cycling cows (progesterone >3 nmol/L, 69.4%) and non-cycling cows (progesterone <3 nmol/L, 30.6%). The samples collected 22–24 days after AI were used for early non pregnancy diagnosis (ENPD) based on progesterone concentration. The accuracy of the method was evaluated by subsequent manual pregnancy diagnosis by rectal palpation. Animals with progesterone concentration <2 nmol/L were categorized as non-pregnant and those with concentration >3.5 nmol/L were categorized as probably pregnant. The accuracy of these predictions was 96.6 and 73.3%, respectively. The data relating to farms, inseminated females, AI technicians and semen batch were analysed using the Artificial Insemination database Application (AIDA). The conception rate to the first service was 24.8% (n=306) and the overall conception rate from 597 services was 28.3%. The interval from calving to first service was 74.9 ± 35.2 days (n=306) and from calving to conception was 141.5 ± 108.1 days. The use of the progesterone assay methodology together with the AIDA computer database were found to be appropriate tools to help farmers to improve the reproductive management and fertility of dairy cattle subjected to AI.

1. INTRODUCTION

Milk and meat production in Tunisia has been fluctuating during the last five years. Some factors influencing this variation such as herd management, nutrition and mastitis have been identified and controlled, while others such as environmental conditions are still uncontrolled. The cattle population in Tunisia is 450 000 females. It comprises two categories, local and crossbred animals (250 000 head) and purebred animals (200 000 head). The use of artificial insemination (AI) in Tunisia started in 1964. During 2003, a total of 171 000 first AI were done, 138 000 on the purebred population and 33 000 on the local and crossbred population.

Since the independence of Tunisia, attempts have been made to increase the production of food needed in the country, particularly milk and meat. Therefore, a stable political and economic environment is essential to promote dairy and beef herds to reach better productivity. However, the main problems relating to poor reproduction in cattle are related to management, nutrition and diseases that reduce their productivity. To improve productivity, the application of hormonal assays can provide a valuable contribution, by increasing knowledge on the reproductive functions of animals from a physiological standpoint.

Radioimmunoassay (RIA) of progesterone [1, 2] and Pregnancy Specific Protein B (PSPB) [3, 4] provide the information necessary to establish the physiological basis for the majority of individual infertility cases in cows. These analyses allow one to determine the frequency of each pathological condition and to pass judgment on the causes of herd infertility. Among other things, results from progesterone assays can be used to determine whether AI has been done at the correct time, assess cyclicity and diagnose non-pregnancy at an early stage.

The objectives of this project were to: (a) improve the quality of AI services in Tunisia, including their management, organisation and training of AI technicians, (b) improve the detection of heat by farmers, (c) use progesterone RIA for early non-pregnancy diagnosis (ENPD), and (d) decrease problems of infertility through identification of causes of reproductive inefficiency and implementation of appropriate measures to rectify them.

2. MATERIALS AND METHODS

2.1. Animals and milk sampling

A total of 924 dairy cows from four sites in Sidi Thabet and the North West of Tunisia were used in this study (Table I). The herds differed based on several characteristics such as number of animals, management and breeding system. Three of them (CFPAEB - Agriculture Professional Training Centre of Bovine Breeding, Agri-Tec and El Amra) were large farms. ODESYPANO (North West Sylvopastoral Development Office) is an organization responsible for providing AI services to small dairy farms in the same area. In this case, we pooled the data from all the small farms under the group ODESYPANO.

TABLE I. THE FARMS AND NUMBERS OF ANIMALS USED IN THIS STUDY

Farms		Number of cows
CFPAEB[a]		109
Agri-Tec		28
ODESYPANO[b]		527
El Amra	Centre 1	169
	Centre 2	91
Total		924

[a]Agriculture Professional Training Centre of Bovine Breeding;
[b]North West Sylvopastoral Development Office

Milk was sampled on the day of AI (day 0), and on days 12 and 22–24 after AI. A total of 2830 milk samples were obtained. The distribution of milk samples by farms and day of sampling is showed in Table II.

TABLE II. DISTRIBUTION OF THE MILK SAMPLES COLLECTED BY FARM AND DAY OF SAMPLING

Farms		Samples			
		Day 0	Day 12	Day 22–24	Total
CFPPAEB[a]		122	107	94	323
Agri-Tec		52	37	32	121
ODESYPANO[b]		530	521	501	1552
El Amra	Center 1	246	194	134	574
	Center 2	84	89	87	260
Total		1034	948	848	2830

[a]Agriculture Professional Training Centre of Bovine Breeding;
[b]North West Sylvopastoral Development Office

2.2. Progesterone measurement

All milk samples (n=2830) were assayed by the self coating RIA method described in the manual distributed by the IAEA [5]. The assay characteristics for milk progesterone determination were studied as follows. The sensitivity was calculated after processing 40 tubes of the zero standard (maximum binding) and the detection limit of the assay was found to be 0.13 nmol/L. The reliability of the assay was assessed by examining its reproducibility on samples with low (A) and high (B) levels of progesterone. Coefficients of variation (precision) were 14.5% and 15.6% for 20 samples A and B respectively.

Consequently, the level of progesterone was determined in all three samples (day 0, 12 and 22–24 after AI) for 924 dairy cows. The number of milk samples for day 0 was higher than the total number of cows used because in some cases the same cow was inseminated several times. Hence progesterone was measured in 1034 milk samples collected on day 0.

The timing of AI was considered appropriate if the progesterone level was less than 1.0 nmol/L and incorrect when the concentration was greater than 1.0 nmol/L. Ovulation was assumed to have occurred when an animal whose progesterone value at AI was less than 1.0 nmol/L had a progesterone concentration of 3.0 nmol/L or greater on day 12. For ENPD, a cow was considered non-pregnant if the progesterone level was less than 2.0 nmol/L, pregnant if the value was greater than 3.5 nmol/L and doubtful when the progesterone was between 2 and 3.5 nmol/L. The accuracy of ENPD was determined by rectal palpation 60 days after AI.

2.3. Artificial Insemination Database Application (AIDA)

Data regarding the management of all females were recorded on a computer using the Artificial Insemination Database Application (AIDA) [6]. The main reproductive parameters for the farms were summarized using AIDA and analyzed in relation to the effects of various factors such as the purpose of keeping cattle, milking system, method of heat detection, time of AI, semen dose, inseminator, mucus discharge and progesterone concentrations.

3. RESULTS

3.1. Progesterone concentrations

Results from measuring progesterone in the milk sample collected on day 0 revealed that 10.1% (n=104) of the cows were inseminated at an improper time (progesterone concentration ≥1 nmol/L). The proportion of such animals varied between the farms (Table III) but the differences were not significant (p >0.05).

TABLE III. INCIDENCE OF LOW AND HIGH PROGESTERONE (P4) CONCENTRATIONS IN COWS AT THE TIME OF ARTIFICIAL INSEMINATION (DAY 0) IN THE DIFFERENT FARMS

Farms		No. of samples	P4 <1 nmol/L	P4 ≥1 nmol/L
CFPAEB[a]		122	106 (86.9%)	16 (13.1%)
Agri-Tec		52	48 (92.4%)	4 (7.6%)
ODESYPANO[b]		530	471 (88.9%)	59 (11.1%)
El Amra	Center 1	246	225 (91.5%)	21 (8.5%)
	Center 2	84	80 (95.0%)	4 (5.0%)
Total		1034	930 (89.9%)	104 (10.1%)

[a]Agriculture Professional Training Centre of Bovine Breeding; [b]North West Sylvopastoral Development Office

Progesterone concentrations at day 12 after AI were evaluated using 948 milk samples from cows that had low progesterone concentration on the day of AI (i.e. presumably inseminated at the proper time). Among these cows, 30.6% (n=290) had less than 3 nmol/L at day 12 and were considered as not having ovulated, indicating that they may have been anoestrous or non-cyclic (Table IV). Ovulation was assumed to have occurred in the rest of the cows (69.4%; n=658). The difference between Agri-tec and the other farms was statistically significant (p <0.05).

TABLE IV. PROGESTERONE (P4) CONCENTRATIONS IN COWS AT DAY 12 AFTER ARTIFICIAL INSEMINATION IN THE DIFFERENT FARMS

Farms		No. of samples	P4 ≥3 nmol/L	P4 <3 nmol/L
CFPAEB[a]		107	75 (70.1%)	32 (29.9%)
Agri-Tec		37	36 (97.3%)	1 (2.7%)
ODESYPANO[b]		521	345 (66.2%)	176 (33.8%)
El Amra	Center 1	194	149 (76.9%)	45 (23.1%)
	Center 2	89	53 (59.6%)	36 (40.4%)
Total		948	658 (69.4%)	290 (30.6%)

[a]Agriculture Professional Training Centre of Bovine Breeding; [b]North West Sylvopastoral Development Office

To confirm these interpretations from progesterone measurement, rectal palpation was done 60 days after AI to determine pregnancy status. Among the cows that were classified as non-cyclic, 36.8% (38 of 103) were found to be pregnant. The concentration of progesterone used for discrimination between presence and absence of luteal activity (3 nmol/L) was therefore reassessed by repeatedly calculating the results based on decreasing levels of progesterone as the value for discrimination, as shown in Table V. The level of 1.5 nmol/L progesterone was therefore selected as the value for discrimination in subsequent analyses.

TABLE V. PERCENTAGE OF COWS CLASSIFIED AS NON-CYCLIC WITH DECREASING LEVELS OF PROGESTRONE AS THE VALUE FOR DISCRIMINATION AT DAY 12 AFTER AI

Category	Progesterone at day 12 after AI			
	<3 nmol/L	<2.5 nmol/L	<2 nmol/L	<1.5 nmol/L
% Cows classified as non-cyclic	28.9 (n=154)	22.5 (n=120)	17.9 (n=102)	14.2 (n=81)
% Cows classified as non-cyclic but found pregnant at rectal palpation	36.8 (n=103)	27.5 (n=80)	24.6 (n=65)	21.7 (n=46)

A total of 848 milk samples collected between 22–24 days after AI from cows that had low progesterone on the day of AI were assayed to establish the accuracy of early non-pregnancy diagnosis (ENPD). The results for the different farms are given in Table VI.

TABLE VI. RESULTS OF EARLY NON-PREGNANCY DIAGNOSIS BASED ON PROGESTRONE (P4) CONCENTRATION IN MILK SAMPLES COLLECTED BETWEEN 22–24 DAYS AFTER AI

Farms		Number of samples	Early non-pregnancy diagnosis		
			Negative (P4 <2 nmol/L)	Doubtful (P4 2–3 nmol/L)	Positive (P4 ≥3.5 nmol/L)
CFPAEB[a]		94	28.7% (n=27)	12.7% (n=12)	58.6% (n=55)
Agri-Tec		32	43.7% (n=14)	6.3% (n=2)	50.0% (n=16)
ODESYPANO[b]		501	42.1% (n=211)	9.6% (n=48)	48.3% (n=242)
El Amra	Center 1	134	39.5% (n=53)	14.6% (n=22)	44.1% (n=59)
	Center 2	87	40.2% (n=35)	18.4% (n=16)	41.4% (n=36)
Total		848	40.1% (n=340)	11.8% (n=100)	48.1% (n=408)

[a]Agriculture Professional Training Centre of Bovine Breeding; [b]North West Sylvopastoral Development Office

The relationship between ENPD done by progesterone measurement and pregnancy diagnosis done by rectal palpation 60 days after AI is given in Table VII. In the first category (ENPD negative), accuracy was superior to 92%. The probability of non pregnancy was more than 0.92 if the progesterone value was strictly inferior to 2 nmol/L. In the second category (ENPD Doubtful), Transrectal palpation allowed to underline that 60.1% of dairy cows were pregnant. Finally, in the third category (ENPD presumed positive), accuracy was around 73%. The probability of pregnancy was 0.73 if the level of progesterone was superior or equal to 3.5 nmol/L.

TABLE VII. COMPARISON OF THE RESULTS FROM EARLY NON PREGNANCY DIAGNOSIS BASED ON PROGESTRONE (P4) CONCENTRATION IN MILK SAMPLES COLLECTED BETWEEN 22–24 DAYS AFTER AI AND THE SUBSEQUENT RESULTS FROM RECTAL PALPATION 60 DAYS AFTER AI

Farms		Early non-pregnancy diagnosis					
		Negative (P4 <2 nmol/L)		Doubtful (P4 2–3 nmol/L)		Positive (P4 ≥3.5 nmol/L)	
Diagnosis from rectal palpation		% Negative	% Positive	% Negative	% Positive	% Negative	% Positive
CFPAEB[a]		96.3	3.7	50.0	50.0	14.6	85.4
Agri-Tec		100	0	50.0	50.0	31.3	68.7
ODESYPANO[b]		91.1	9.9	33.3	66.7	25.3	74.7
El Amra	Center 1	96.2	3.8	41.0	59.0	37.3	62.7
	Center 2	100	0	25.0	75.0	25.0	75.0
Total		96.6	3.4	39.9	60.1	26.7	73.3

[a]Agriculture Professional Training Centre of Bovine Breeding; [b]North West Sylvopastoral Development Office

3.2. Artificial Insemination Database Application

All data concerning the management of the three large dairy farms (i.e. except ODESYPANO) and information related to 446 inseminated cows were recorded using the AIDA computer database. The conception rate to the first service was 24.8% (n=306) and the overall conception rate from 597 services was 28.3%. The interval from calving to first service was 74.9 ± 35.2 days (n=306) and from calving to conception was 141.5 ± 108.1 days (n=105). Table VIII shows the conception rates in the different farms.

TABLE VIII. CONCEPTION RATES (CR) TO THE FIRST SERVICE AND TO ALL SERVICES IN THREE LARGE FARMS

Farms	First service		Overall	
	Number	CR (%)	Number	CR (%)
CFPAEB[a]	57	35.1	94	42.6
Agri-tec	7	14.7	59	23.7
El Amra Center 1	106	27.4	254	28.5
Center 2	66	39.4	96	45.8

[a]Agriculture Professional Training Centre of Bovine Breeding

The interpretation of data from progesterone measurement in the different combinations of milk samples was done using the AIDA computer database. The results from all three successive milk samples followed by rectal palpation are shown in Table IX.

TABLE IX. INTERPRETATION OF RESULTS FROM PROGESTRONE CONCENTRATION IN THREE MILK SAMPLES (DAYS 0, 10–12 AND 22–23 AFTER AI) AND SUBSEQUENT RECTAL PALPATION

Day 0 (AI)	Day 10 – 12	Day 22 – 23	Pregnancy Diagnosis	Frequency (n)	Interpretation
Low	High	High	Positive	**79 (29.2%)**	Pregnant
Low	High	Low	Negative	**26 (9.6%)**	Non fertilization, early embryonic mortality, post AI Anoestrus
Low	High	High	Negative	**28 (10.3%)**	Late embryonic mortality (>day 16), Luteal cyst, persistent CL
High	High	High	Positive	**0**	AI on pregnant animal
*	*	*	Positive/ Negative	**106 (39.1%)**	* At least one of the samples showed an intermediate value (1 to 3 nmol/L). Other clinical data is required for proper interpretation
		Total occurrence		**271**	

4. DISCUSSION

4.1. Progesterone concentrations

The success of AI is greatly influenced by the heat detection system in the herd and the timing of artificial insemination. This study showed that 10.1% of dairy cattle were inseminated at an improper time, when the progesterone levels were more than 1 nmol/L. The highest incidences of incorrect timing were observed at the CFPAEB (13.1%) and ODESYPANO (11.1%) farms. This variation was probably due to the consequence of herd management and the heat detection method. According to some reports [7, 8] 10% can be considered acceptable and, although others [9, 10] have reported lower incidence, the findings of the present study are comparable to those reported in many studies [11–14].

The determination of sexual activity using progesterone measurement in two milk samples at an interval of 12 days enabled the detection of cyclic cows in which ovulation had not occurred. The incidence in this study was relatively high (30.6%) and the major factors responsible of this appear to be herd management and nutrition. This hypothesis was reinforced by the finding that there was no significant difference between CFPAEB, El Amra and ODESYPANO, where the management and the nutrition of cattle were similar. But a significant difference ($p < 0.05$) was noted between these farms and Agri Tec and this could be attributed to the smaller number of animals and the better nutrition in the latter herd.

According to the progesterone values at day 12 after AI and the subsequent rectal palpation, 36.8% of cows initially classified as not-cyclic were found to be pregnant. Thus the level of progesterone used for discriminating between the presence and absence of luteal activity was re-examined and the threshold was lowered. In spite of this, 21.7% of cows were still classified as non-cyclic, which is unexpectedly high. The reasons may be misidentification of cows or milk samples, and conception to a subsequent repeat AI which was not recorded.

The results from the milk samples collected 22–24 days after AI were used to standardize the criteria for ENPD and to determinate its practically under Tunisian conditions. The conclusion was that a cow having less than 2 nmol/L of progesterone 22–24 days after AI can be considered to be non-pregnant. This was confirmed in 96.6% of cases by subsequent rectal palpation. On the other hand, a cow having more than 3.5 nmol/L of progesterone at this time can be presumed to be pregnant, but this was confirmed only in 73.3% by rectal palpation. These results are comparable to many previous studies in several countries [14–18].

4.2. Artificial Insemination Database Application

A common problem faced by the majority of owners of dairy cattle herds is the recording and analysis of data concerning reproduction and fertility. The AIDA computer database was found to be a very useful tool for this purpose and enabled the identification of problem areas in reproductive management, thereby helping to develop appropriate solutions to improve reproductive performance.

The findings of this study indicate that fertility in the farms is low, with average first service conception rate of 24.8% and overall conception rate of 28.3%. The mean interval from calving to first AI was 74.9 ± 35.2 days and from calving to conception was 141.5 ± 108.1 days. These intervals are about 5 and 42 days, respectively, longer than the target intervals needed to obtain one calf per cow per year [19]. The overall fertility indices were comparable to [20, 21] or better [22] than those reported previously.

The analysis of effects of farm, parity, body condition score, inseminator and milk production on reproductive parameters showed no significant relationships ($p < 0.05$).

4.3. Impact assessment

The impact that results from the implementation of this project under Tunisian conditions is not in any doubt. This study allowed the identification of two main inefficiencies in AI, namely the high percentage of females being inseminated (a) at an improper time (due to poor heat detection) and (b) without sexual cyclicity. It shows the economic advantages of using progesterone measurement for ENPD and to diagnose certain individual infertility problems. It also provided accurate information on the reproductive performance of the farms studied, allowing comparison with previously published data and pointing out areas where appropriate measures need to be taken to improve reproductive efficiency.

Accordingly, the management of reproduction and breeding can be improved by ensuring proper conditions at the time of AI. This includes correct detection of heat and the availability of the AI technician at the correct time in relation to the onset of heat. After AI, the routine use of progesterone measurement for ENPD will help the farmer and to improve reproductive performance of his cows.

4.4. Conclusions and recommendations

The implementation of this project in Tunisia has been successfully carried out, using progesterone measurement and the AIDA computer database as tools. The results have shown that the fertility of dairy herds is low and an analysis of various factors has identified the major reasons for this situation. This will help in finding appropriate methods to improve reproductive performance and thereby increase the productivity of dairy cattle.

It is necessary to continuously provide farmers with information on the importance of improved management practices, especially feeding and heat detection. It is also necessary to sensitize AI technicians on the importance of ensuring that a cow is on heat at the time of AI and on adopting the correct technique of performing AI. Further, veterinarians need to be sensitized on the need to perform pregnancy diagnosis by rectal palpation as a routine measure on all animals subjected to AI and not returning to heat by 60 days.

In order to ensure these activities are carried out, the Government has to arrange regular refresher and training courses for AI technicians and provide financial incentives for the veterinarians to perform pregnancy diagnosis. The ENPD service should also be initially supported by the Government and become self-supporting with a gradual scheme of cost-recovery.

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USE OF MILK PROGESTERONE MEASUREMENT TO IMPROVE DAIRY CATTLE PRODUCTION IN ZAMBIA THROUGH EARLY DETECTION OF INFERTILITY AND NON-PREGNANCY

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Abstract

Reproductive efficiency among various cattle breeds in Zambia is reported to be very low, as evident from the long calving intervals and low number of offspring born in the lifetimes of each cow. However, the factors responsible for low reproduction have not been fully studied. The objectives of this study were to improve the efficiency of artificial insemination (AI) through the provision of a diagnostic service for early detection of non-pregnancy and identification of causes of infertility. Milk samples were collected on day 0, 10–12, and 22–24 after AI for progesterone analysis by radioimmunoassay (RIA). Survey information was recorded in a computer using the Artificial Insemination Database Application (AIDA) and analysed for various reproductive parameters. The diagnostic service had accuracies for predicting and detecting pregnancy of 96 and 93%, respectively. Over 90% of cows were presented for AI when progesterone was low and only 4% had high progesterone, indicating that errors in oestrus detection were low. There was significant ($P<0.04$) variability among farms with regard to the intervals from calving to first AI and conception and the pregnancy rates. Pregnancy rates were significantly influenced by the purpose of keeping cattle ($P <0.01$), type of housing ($P <0.03$), method of keeping records ($P <0.02$) and season of calving and insemination ($P <0.01$). The farmers and AI technicians made very little use of some vital signs of heat. The diagnostic service was very accurate and reliable in detecting various reproductive states and was well received among farmers.

1. INTRODUCTION

Livestock production in Zambia forms part of the livelihood for a number of tribes in the traditional cattle keeping areas of Eastern, Southern, and Western Provinces where livestock are seen as a living bank that also serves as a source of draught power, milk and meat as well as manure for improving soil quality. However, cattle numbers are also high on large farms in Lusaka and Central Provinces.

Commonly, three types of livestock farming systems are encountered, namely commercial, emergent and traditional. Typically, commercial farms are characterized by large herds kept under appropriate housing, optimal feeding and good management systems, with owners showing desire to increase productivity. At the other end of the spectrum are traditional farmers who have large herds of cattle that generally roam about in communally grazed land and virtually scavenge for part of the year when both quality and quantity of feed become limiting. Very little production inputs are practiced although the will to improve animal productivity abounds. In between these two production systems are the emergent farmers who keep fewer animals, generally in enclosed kraals with minimal inputs in terms of medication, manual labour and feed, and adopt less than optimal management practices but also wish to improve the productivity of their animals.

Artificial insemination (AI) in Zambia started in the early 1950's using locally produced fresh semen collected from high grade Friesian bulls. For almost ten years, the application of AI remained low. However, with the realization of the critical role that AI stood to play in the overall improvement of cattle production, a National AI Service (NAIS) was introduced through which good quality semen was provided to small-scale farmers. Since then, AI has continued to grow in popularity and use, especially among the commercial farmers, whose cattle constitute 18% of the 2.4 million national population. Of these, 120 000 are dairy animals and nearly 75% of them are bred using AI. Currently, Zambia imports about 7540 straws of frozen semen per year. The use of AI among small scale and emergent dairy farmers is not very popular, however, largely due to perceived high input costs, lack of technical know-how on practices such as timing on AI, poor results arising from poor heat detection, and poorly organized communication with AI service providers.

The problems experienced by farmers are exacerbated by the fact that nearly 40% of local breeds of cattle indigenous to Zambia have been reported to be infertile [1]. The primary cause of the infertility is reported to be poor nutrition, which results from a decline in both quality and quantity of available fodder as the weather conditions change from the wet to dry seasons. Early signs of this deficiency include an increase in the incidence of inactive ovaries and silent oestrus that has been reported to occur between May and October [1]. Additionally, low reproductive rates in cattle are predisposed by both anatomical and physiological factors (prevalence of inactive ovaries and low progesterone values, respectively), which indicate that ovarian activity resumes in December [2]. Furthermore, severe under-nutrition may result in the absence of follicles greater than 3–4 mm in diameter and affects the regulation of size, dynamics and growth of dominant follicles in cattle [3].

In previous research conducted in Zambia, neither feed supplementation packages nor use of short-term calf removal to induce early resumption of ovarian activity were able to significantly improve the performance of local breeds of livestock [2]. Consequently, it was deemed essential that other methods for resolving the prevailing low efficiency of reproduction in cattle be found and used. Notable among these was the need to improve the efficiency of AI, to ascertain the appropriate time of doing AI, to detect cows that are anoestrus or have cystic ovaries, and to conduct pregnancy diagnosis by 22 days after AI.

Therefore, the objective of the current study was to improve the efficiency of AI using a diagnostic service for dairy farmers based on progesterone radioimmunoassay (RIA) by: (i) assessing the current status and identifying constraints to AI; (ii) formulating and implementing remedial measures to identified constraints; (iii) establishing a routine service to dairy farmers for early diagnosis of non-pregnancy, infertility, and proper timing of heat; and (iv) establishing the cost-benefits associated with such a service. The intended outcome was improved household food security by increasing meat and milk production and the profitability of dairy farming.

Since there were no reports on the factors that deleteriously affect reproduction in dairy cattle in Zambia, farms from all dairy production sectors were included in the study.

2. MATERIALS AND METHODS

2.1. Study location and farming systems

The study, which covered the period 1999–2004, was carried out in five districts (Figure 1) where the elevation above sea level was 1070 to 1370 metres and annual rainfall ranged from 762 to 1200 mm. The ambient temperature in the study area ranged from 17.2°C in July, the coldest month of the year, to 30°C in October, which is the hottest month of the year. The districts, farming systems and farms used in the study are given in Table I.

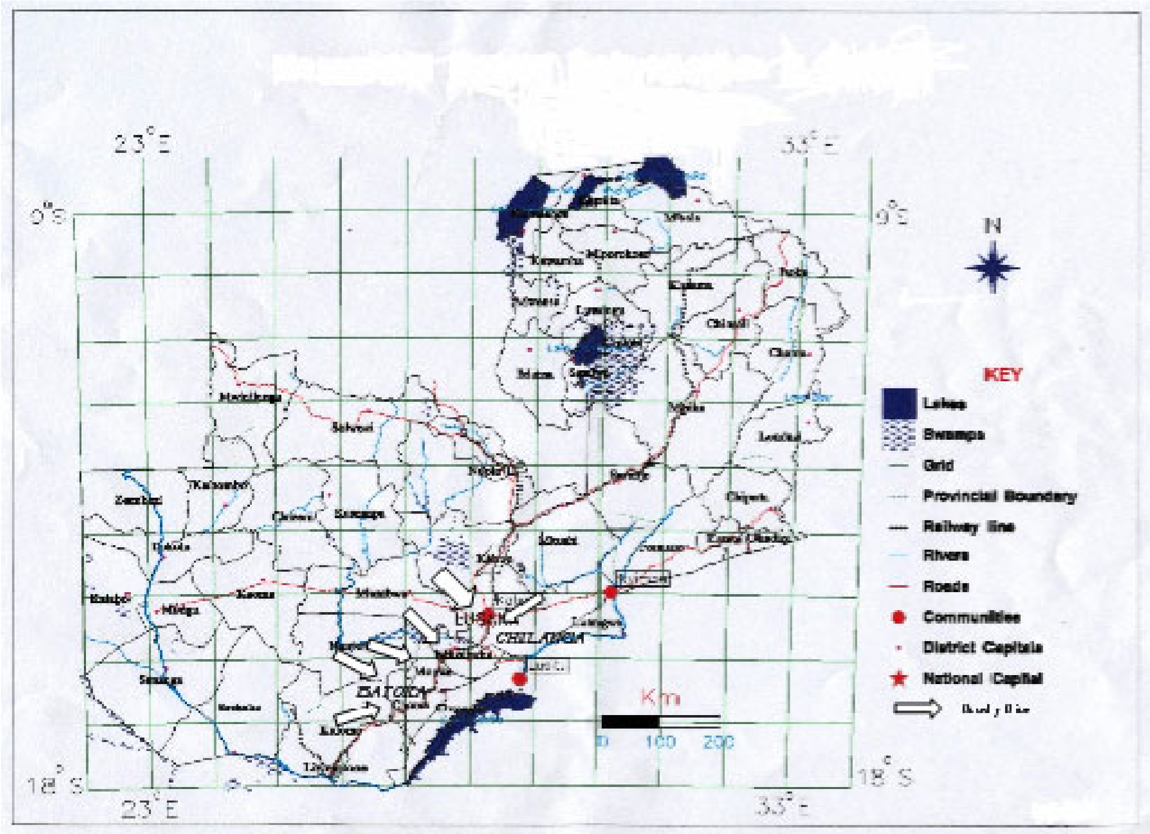

FIG. 1. Map of Zambia showing districts (⇨) from which farms were selected for participation in the study.

TABLE I. DISTRICTS, FARMING SYSTEMS AND FARMS INCLUDED IN THE STUDY

District	Farming system	Farm(s)
Batoka	Emergent	Livestock Development Centre (LDC), Golden Valley Agricultural Trust (GART)
Chilanga	Emergent	Miller
	Commercial	Zamdael
Lusaka	Small Scale	Divecha, Mazuru, Rosedale
	Emergent	Agriflora, Palabana Dairy Training Institute, Witham
	Commercial	Sunrise
Monze	Emergent	Harridon
Mazabuka	Commercial	Chisoba, Kushiya, Simonga, Syringa
	Small Scale	Milambo & Brothers (M&B)

The farmers reported keeping information on reproductive health and general management using individual cow cards, breeding calendars or on computers. Most farmers kept information on individual cow production. At least one farmer was not keeping any written records, however.

Generally, animals were housed in corrals or night paddocks with few using loose barns. Cows ranged from first to ninth parity and were largely kept for dairy purposes but some animals were used for beef and draught. The draught animals were not necessarily the same that were kept for milking purposes. The cows were mainly Friesian-Holsteins and a few Jerseys. Some of the farmers had crosses arising from the use of Friesian-Holstein and Jersey bulls on cows of various local breeds. The cows were milked twice a day either by hand or machine. Most animals were fed a diet that included grazing, concentrate and roughage (GCR), some were fed on roughage and concentrate (RC); grazing and concentrate (GC); or grazing and roughage (GR). Heat was detected either visually by herdsmen or using teaser animals. The nature or type of calving was recorded with the categories being normal calving or calving with simple or major assistance while those with retained placenta were noted as such. No routine pregnancy diagnosis (PD) was done on at least two farms while most farmers conducted PD by rectal palpation between 60–120 days after AI.

All cows were inseminated by 27 AI technicians (AITs) using semen in either 0.25 or 0.50 ml frozen French straws imported by a private company based in Lusaka. The semen was from 130 Friesian, Jersey and Boran bulls. Each farm employed either one or more AITs who operate on a do-it-yourself (D-I-Y) basis. The only exception was the Mazuru dairy farm, which was served by the AIT from the Palabana Dairy Training Institute, which operates as a private farm under the Livestock Development Trust. The total number of AIs conducted during the study period was 1219 (Friesian=1087, Jersey=28, Boran=10), of which 703 were first AIs.

2.2. Data sampling

The study was conducted in collaboration with the Ministry of Agriculture and Cooperative's NAIS and Parmalat, a private milk processing company. In order to establish the current status of AI as practiced in Zambia, a questionnaire-based survey was carried out at the collaborating institutions and farms. The questionnaire captured information regarding the farm, the AIT, the bull and the cow, with each parity and service being recorded separately.

The accuracy or efficiency of the farmers' ability to detect animals in true oestrus was established using milk samples collected on day 0 (day of AI), and on days 10–12 and 20–22 after AI. The latter two samples were also used to determine whether or not an animal ovulated, was returning to oestrus, or both. Samples were collected by farmers at milking in sample vials with a preservative (sodium azide).

The samples were centrifuged at 2000 *g* and the skim milk that was separated was stored at -20°C until analysis for progesterone was conducted using the IAEA/FAO 'Self-coating' RIA system [4]. The inter-assay coefficients of variation (CV) for internal quality control samples containing high (15.6 ± 1.5 nmol/L), medium (6.01 ± 0.4 nmol/L) and low (0.66 ± 0.1 nmol/L) concentrations of progesterone measured in 11 assays were 9.6, 7.0, and 15.0%, respectively. The corresponding intra-assay CVs for high, medium, and low samples ranged from 0.16–2.32, 0.23–2.29, and 0.51–2.62%, respectively.

2.3. Data analysis

Data were initially captured and processed using the Artificial Insemination Database Application (AIDA), which was developed by the FAO/IAEA [5]. Data was further processed and analysed in SAS® [6] using PROCEDURES Means, FREQ and GLM, as appropriate. Unless stated otherwise, results are presented as mean and the associated standard deviation (Mean ± STD). Data were analysed using variants of the following model:

$$Y=\mu+St+F+P+Cs+As+Sv+e;\text{ where}$$

- Y=dependent variable (Progesterone on day 0, 10-12, & 22-24; days from calving to AI, days from calving to conception);
- St=the effect of status (pregnant versus non-pregnant);
- F= the effect of farm (all 18 farms);
- P= the effect of parity (1–8);
- Cs= the effect of calving season (hot-dry, cold-dry, wet);
- As= the effect of AI season (hot-dry, cold-dry, wet);
- Sv= the effect of service; and
- e=residual error.

Where main effects were significantly different ($P \geq 0.05$), means were separated using multiple range tests or frequency distribution, whichever was appropriate. For all categorical data, non-parametric analyses was applied using two-way tables, such as status x farm; status x suckling; status x AI season; status x calving season, etc.

3. RESULTS

3.1. Progesterone

Progesterone analysis was conducted in all 2448 samples received at the RIA laboratory in Chilanga. Progesterone levels were categorized as 'low' (<1 nmol/L), 'intermediate' (1–3 nmol/L) or 'high' (>3 nmol/L). The number of animals with levels of progesterone falling in these categories is expressed as a percentage of the total number of animals for that category. In 92% of the 1053 cows presented for AI, progesterone levels were low, indicating that AI was probably done at the correct time. In 3.5% of cows, AI was done when progesterone was high. The rest of the cows (4.4%) had progesterone levels that were intermediate. In the second sample collected on days 10–12 after AI, 66.8% of the cows had high levels, indicating that they had ovulated, while in 14.4% of the cows the levels were low, indicating that they were anoestrous, anovulatory or had short luteal phases lasting less than 10 days. Of the cows that were presented for AI when progesterone was high, 1.3% also had high levels at the second sampling, indicating that they were likely to have been pregnant at the time of AI.

There was a significant ($P < 0.001$) effect of farm for progesterone level at day 0 and day 10–12. For day 0, this significant variation was affected by a significant ($P < 0.001$) interaction between farm and the season when the AI was conducted.

Data from 189 cows was used to ascertain pregnancy status in the farms covered in the study. The results from the assay correctly predicted non-pregnancy at 21 days after AI with an accuracy of 96%, and detected 93% of the animals that were later confirmed as pregnant. The interpretation of results based on the progesterone values at the three stages of sampling and the results of manual PD conducted 6–120 days after AI are summarized in Tables II and III.

TABLE II. CONCURRENCE BETWEEN MILK PROGESTERONE NON-PREGNANCY TEST BASED ON THREE SAMPLES COLLECTED ON DAYS 0, 10–12, AND 22–24 AFTER AI AND PREGNANCY DIAGNOSIS CONDUCTED BY RECTAL PALPATION AT DAYS 60–120 OF GESTATION

Day 0	Day 10–12	Day 22–24	Pregnancy Diagnosis	Frequency n (%)	Interpretation
Low[a]	High[b]	High	Pregnant	84 (44.4)	AI at ovulatory oestrus and pregnant
Low	High	Low	Non-Preg	3 (1.6)	Fertilization failure, early embryonic mortality, post-AI anoestrus
Low	High	High	Non-Preg	6 (3.2)	Late embryonic mortality (>day 16), Luteal cyst, persistent CL
High	High	High	Pregnant	3 (1.6)	AI on pregnant cow
Low	Low	Low	Non-Preg	1 (0.5)	Anoestrus, follicular cyst
*	*	*	Preg/Non-P	58 (30.7)	At least one sample had intermediate value
?	?	?	Preg/Non-P	34 (18)	Results from progesterone and PD do not correlate (errors in sampling or assay, problem with management or cow)
Total				189 (100)	

[a]Low = <1 nmol/L; *Intermediate = 1–3 nmol/L; [b]High = >3 nmol/L

TABLE III. CONCURRENCE BETWEEN MILK PROGESTERONE NON-PREGNANCY TEST BASED ON TWO SAMPLES COLLECTED ON DAY 0 AND 22–24 AFTER AI AND PREGNANCY DIAGNOSIS CONDUCTED BY RECTAL PALPATION AT 60–120 DAYS OF GESTATION

Day 0	Day 22–24	Pregnancy Diagnosis	Frequency n (%)	Interpretation
Low[a]	High[b]	Pregnant	123 (60.3)	AI at ovulatory oestrus and pregnant
Low	Low	Non-Preg	4 (2.0)	Fertilization failure, early embryonic mortality, post-AI anoestrus, cystic
Low	High	Non-Preg	8 (3.9)	Late embryonic mortality (>day 16), Luteal cyst, persistent CL
High	High	Pregnant	7 (3.4)	AI on pregnant cow
High	Low	Non-Preg	0 (0)	AI during luteal phase with subsequent unobserved oestrus
*	*	Preg/Non-P	42 (20.6)	At least one sample had intermediate value
?	?	Preg/Non-P	20 (9.8)	Results from progesterone and PD do not correlate (errors in sampling or assay, problem with management or cow)
Total			204 (100)	

[a]Low = <1 nmol/L; *Intermediate = 1–3 nmol/L; [b]High = >3 nmol/L

3.2. Reproductive health management by farmers

Data on the effect of various management parameters is presented in Table IV and Figure 2. The interval from calving to first service (AI) was 118 ± 88 days (range 53–365 days) and the interval from calving to conception was 127 ± 80 days (range 69–347 days). There was a significant (P <0.04) effect of farm on both intervals. However, there was also a significant (P <0.03) interaction between farm and season when the AI was done.

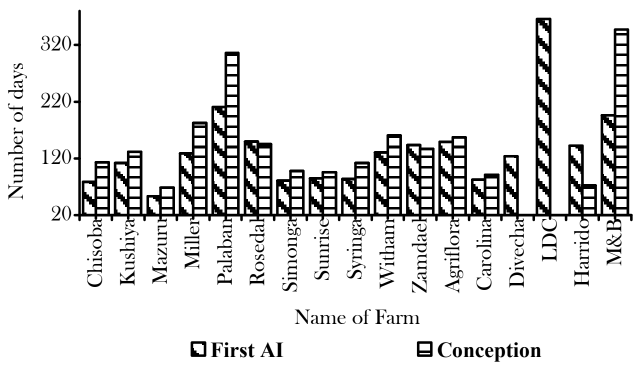

FIG. 2. Effect of farm on the interval from calving to first service and to conception among dairy cows (P <0.01). Note: LDC = Livestock Development Centre, M&B = Milambo & Brothers; No conception data was available for Divecha and LDC dairy farms.

TABLE IV. THE EFFECT OF VARIOUS MANAGEMENT PARAMETERS ON THE INTERVAL FROM CALVING TO FIRST SERVICE AND TO CONCEPTION AMONG DAIRY COWS

Parameter	Interval (days) from calving to			
	First service		Conception	
	n	Mean ± SD	n	Mean ± SD
Purpose:				
Dairy	418	119.8 ± 75.0	269	135.0 ± 77.0
Dairy/Beef	104	96.8 ± 49.0	61	121.0 ± 62.3
Dairy/Draught	115	93.0 ± 99.1	67	107.0 ± 99.0
Dairy/Beef/Draught	65	180.0 ± 147.6	2	73.0 ± 6.0
Housing:				
Corral/Paddock	207	127.0 ± 75.5	141	134.0 ± 70.0
Loose Barn	11	365.0 ± 209.0	-	-
Night Paddock	474	107.0 ± 80.0	255	124.0 ± 85.0
Heat Detection:				
Teaser	214	98.7 ± 49.5	134	114.4 ± 60.2
Visual	478	125.8 ± 100.0	262	134.1 ± 87.8
Feeding System[a]:				
GCR	480	118.7 ± 86.0	289	123.3 ± 65.8
GC	29	171.7 ± 119.2	20	234 ± 181.6
GR	34	129.1 ± 68.0	17	182.6 ± 81.9
RC	149	100.1 ± 61.6	70	100.7 ± 57.0
Record Keeping:				
Breeding calendar	82	172.9 ± 134.0	30	145.6 ± 94.9
Computer	422	93.9 ± 46.7	270	111.8 ± 54.5
Cow cards	180	143.7 ± 93.6	93	159.9 ± 96.4
No written record	8	195.8 ± 338.9	3	347.3 ± 385.3
Type of calving:				
Retained Placenta	26	168.7 ± 104.1	13	136.0 ± 80.7
Major assistance	6	147.3 ± 93.0	5	231.0 ± 61.0
Simple assistance	48	93.2 ± 46.9	32	119.3 ± 71.0
Normal	613	117.2 ± 89.9	344	126.0 ± 79.6

[a]G = grazing, C = concentrates, R = roughage

3.3 Reproductive parameters

Data on the effect of various parameters on conception rate (CR) to first service and all services are presented in Table V and Figures 3–5. The observed CRs were significantly influenced by farm (χ^2, P <0.001, Figure 6). Similarly, there was a significant influence of the following factors on CRs: purpose for keeping cows (χ^2, P <0.01); type of housing used (χ^2, P <0.03); method of record keeping on the farm (χ^2, P <0.02); season in which the AI was done (χ^2, P <0.02); and season of the calving immediately preceding the AI.

TABLE V. THE EFFECT OF VARIOUS MANAGEMENT PARAMETERS ON CONCEPTION RATES TO FIRST SERVICE AND ALL SERVICES AMONG DAIRY COWS

Parameter	Conception rate						Services per conception
	First service			All services			
	Cows (n)	Preg (n)	Preg (%)	Cows (n)	Preg (n)	Preg (%)	
Purpose:							
Dairy	419	163	38.9	770	269	34.9	2.9
Dairy/Beef	104	34	32.7	209	61	29.2	3.4
Dairy/Draught	115	57	49.6	148	67	45.3	2.2
Dairy/Beef/Draught	65	2	3.1	92	2	2.2	n/a
Housing:						-	
Corral/Paddock	208	84	40.4	390	141	36.2	2.8
Loose Barn	11	0	0	11	-	0	n/a
Night Paddock	474	171	36.1	801	255	31.8	3.1
Heat Detection:							
Teaser	214	90	42.1	357	134	37.5	2.7
Visual	479	165	34.4	845	262	31.0	3.2
Feeding System[a]:							
GCR	481	171	35.6	899	289	32.1	3.1
GC	29	11	37.9	62	20	32.3	3.1
GR	34	12	35.3	41	17	41.5	2.4
RC	149	61	40.9	200	70	35.0	2.9
Record Keeping:							
Breeding calendar	82	27	32.9	117	30	25.6	3.9
Computer	422	162	38.4	794	270	34.0	2.9
Cow cards	181	66	36.5	264	93	35.2	2.8
No written record	8	0	0	27	3	11.1	9.0
Type of calving:							
Retained Placenta	26	11	42.3	312	13	40.6	2.5
Major assistance	6	2	33.3	12	5	41.7	2.4
Simple assistance	48	18	37.5	87	32	36.8	2.7
Normal	613	222	36.2	1074	344	32.0	3.1
Time of AI:							
AM	268	94	35.1	476	158	33.2	3.0
PM	435	162	37.2	743	241	32.4	3.1
Mode of Milking:							
Hand	367	122	33.2	618	180	29.1	3.4
Machine	326	133	40.8	584	216	37.0	2.7

[a]G = grazing, C = concentrates, R = roughage

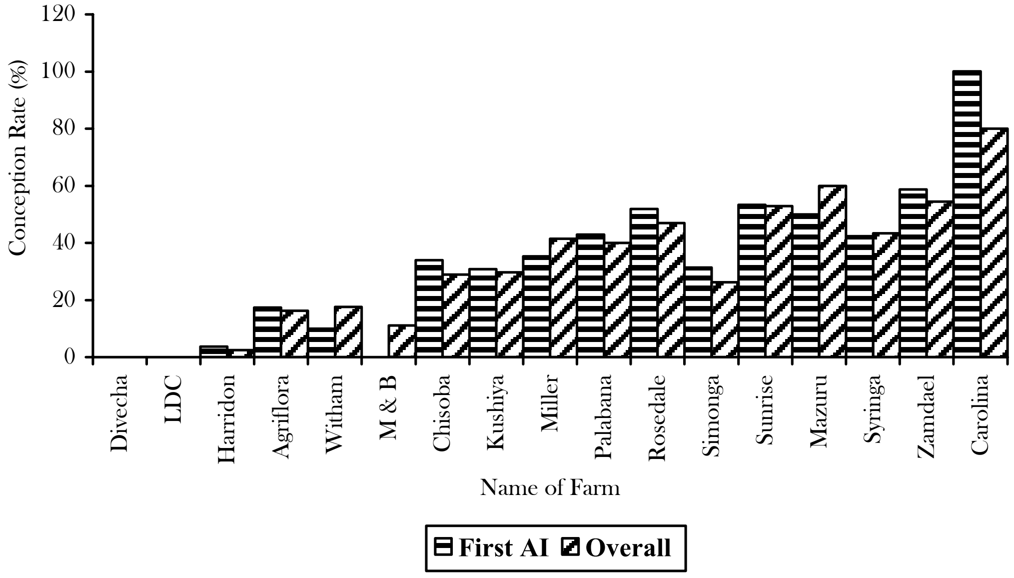

FIG. 3. Effect of farm on conception rates to first and all services among dairy cows. (farm, χ^2, $P<0.001$)

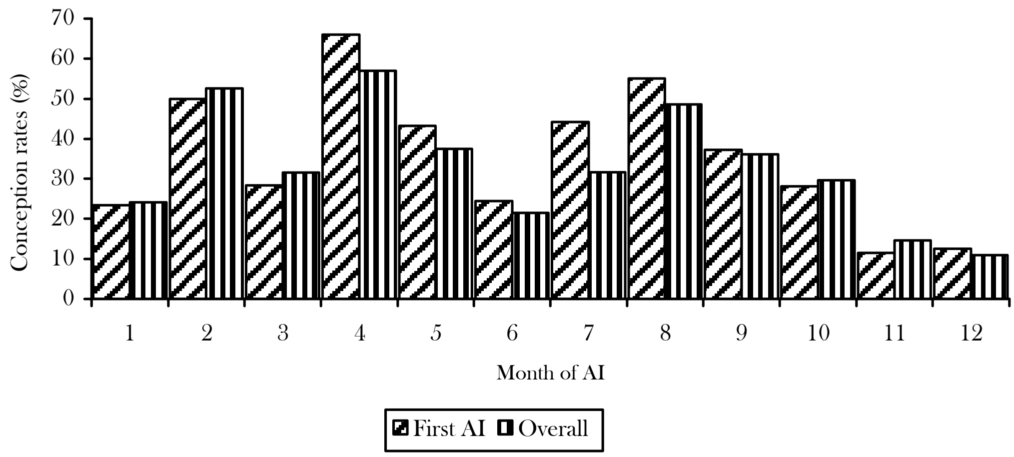

FIG. 4. Effect of month when AI was done on the conception rates to the first and all services among dairy cows (χ^2, $P<0.02$).

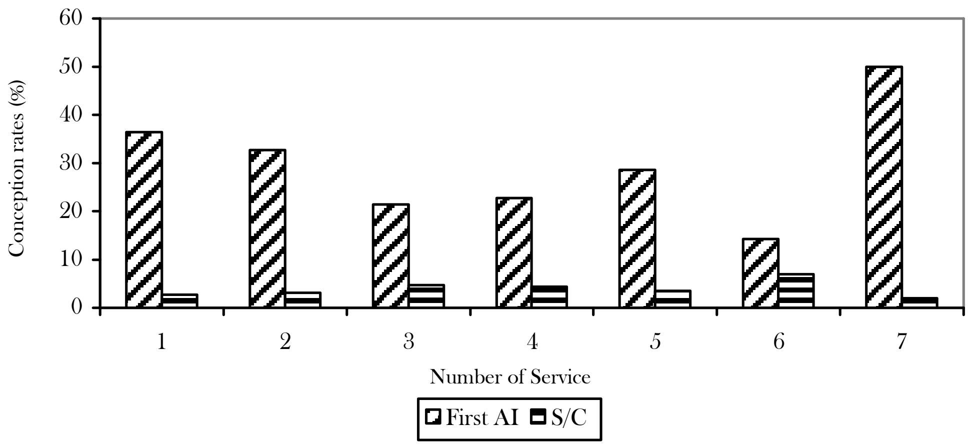

FIG. 5. The effect of service number on conception rates and on the number of services per conception (S/C) among dairy cows.

Factors relating to the cow, the oestrous period, the inseminator and the performance of that particular AI were recorded for 1219 services, of which 709 were first services after calving. The CRs to first and all services in the different categories are given in Table VI.

The overall CR for cows whose heat signs at AI were bellowing (n=11), mounting other cows (n=25) and standing to be mounted by other cows (n=1167) were 18.2, 16.0, and 33.3 %, respectively. No cows conceived when only mucus discharge (n=2) or other subsidiary signs (n=9) were the basis of heat detection.

TABLE VI. THE EFFECT OF VARIOUS FACTORS RELATING TO THE COW, OESTROUS PERIOD AND THE INSEMINATOR ON CONCEPTION RATES TO FIRST SERVICE AND ALL SERVICES AMONG DAIRY COWS

Parameter	Conception rate (%)	
	First service	Overall
Vulval swelling:		
Marked	42.7	39.3
Slight	31.1	28.5
None	35	30.5
Mucus discharge:		
Clear	41.5	37.1
Purulent	0	0
Turbid	4.1	3.9
None	27.4	27.1
Uterine tone:		
Marked	40	33.7
Slight	29.6	28.8
None	39.4	34.9
Control of oestrous cycle:		
None (natural heat)	37.1	33.2
Synchronized heat	26.7	22.2
Suckling by the calf:		
None	37.3	32.7
For milk let-down only	31.5	30.3
Several hours/day	40.2	40.5
Education of AI technician:		
Primary school	35.6	25.9
High school	28.4	32.7
College, diploma	47.9	43.8
University, degree/	41.2	46.2
Experience of AI technician:		
< 10 years	35	32.6
10–19 years	44.6	38.8
>20 years	54.8	49.1
Site of semen deposition:		
Cervix	41.7	35.6
Vagina	36.1	33.3
Uterus	36.1	32.6
Ease of passing the pipette through cervix:		
Easy	36.8	32.9
Difficult	10.0	23.1

The mean interval between first and second service was 51.06 ± 40.66 days (n=72) and ranged from 21 to 123 days among the farms. There were few observations (n=29) recording the interval between second and third services, the average being 28.4 ± 12.8 days (range 18–50 days). The inter-service interval was influenced by the method of heat detection used, being 45.3 ± 26.5 days when heat was detected using a teaser and 54.0 ± 45.5 days when it was by visual observation.

4. DISCUSSION

The AIDA computer software, which was developed by IAEA/FAO for capturing, storing and preliminary analysis of information related to AI proved to be an excellent tool for this study. It greatly facilitated the process of making decisions due to its ease of use and ability to make information available instantly. The farmers who used computers for storing and managing information achieved much better results with regard to time related fertility parameters.

Various technologies exist that detect or aid in the detection of factors that predispose cattle to losses in reproduction. These include highly sensitive diagnostic tools such as RIA and enzyme-linked immuno-sorbent assay (ELISA) to measure progesterone concentrations. These techniques can be used on various biological materials from species such as cattle [7], pigs [8] and even lizards [9]. Consistent with these reports, the results obtained in the present study indicate that the 'self-coating' RIA developed by the IAEA/FAO can serve as a reliable means for providing a sustainable early non-pregnancy diagnosis service to farmers. Such a service would be more preferred in relation to others such as rectal palpation that are not only done much later but have also been associated with an increase (9.3%) in embryo loss [10].

The accuracy of 93–96% to either detect or predict conception found in this study is similar to previous reports [11]. Such a tool is very good in that it offers answers three to four oestrous cycles before the earliest possible time with the conventional method of rectal palpation. The results presented herein also show that various reproductive parameters vary considerably from one farm to another. Notable among these variations is the long interval taken after calving to show detectable heat resulting in the first service. The intervals are longer among small scale than commercial dairy farmers. This is consistent with previous reports [12] that linked this extended duration to phyto-oestrogens of environmental origin [13]. This extension resulted in calving intervals that are longer than normal, a problem that has been a subject of many unsuccessful attempts at resolving.

The finding that the duration to the first service and conception varied differently during the year confirms previous reports [14] suggesting that this could be attributable to the variations in the quality and quantity of available feed. Normally, good grazing is available between the period December to May, after which the lack of rain decreases the quantity and quality of pasture. The fluctuations after this stage seen in the data could be attributed to attempts by farmers to meet the requirements of the animals through supplementary feeding.

The great variation in the interval between services, from 22 to as much as 123 days, shows that farmers are less likely to keenly observe a cow for oestrus after it has been served. This notwithstanding, the observation that the interval varies widely could be also due to the failure by farmers to follow and present milk samples for assay from each animal in subsequent services. This tendency repeats itself for several cycles, resulting in a number of missed heats.

The present survey shows CRs that are similar to or even higher than those reported by others [15]. It is apparent, however, that the values reported in the current survey are influenced by the failure on the part of the project team or the farmer to consistently collect and record clinical observations on each animal. Farmers were more interested in knowing what the laboratory results were, than in reporting the oestrus status of animals for which they submitted samples. The obvious consequence of this is the artificial distortion of the calving and inter-oestrus intervals reported. The failure to record all returns to oestrus resulted in lower overall CRs than would have been the actual case. Therefore, it is imperative that all, or at least as much data as possible, is collected and analysed.

The finding in this study of higher CR in some animals that were reported as showing no overt signs of heat compared with those that were reported as showing mild signs is contrary to previous reports and accepted principles. This suggests that either the AIT's did not understand the role of these physiological parameters or that they failed to report the actual name of the organ involved, possibly for cultural reasons. For example, when asked to state the site of depositing semen, farmers and AITs found it difficult to differentiate between the vagina and uterus, or simply used the two terms interchangeably. Obviously, this would lead to the erroneous conclusion that one can place semen in the vagina and still obtain acceptably high CR. Therefore, more in-service training is needed for the AITs and dairy farmers, including basic training in reproductive anatomy and terminology.

Considerable difficulty was experienced in computing some reproductive health parameters simply because there was no binding agreement to ensure that each animal recruited for the study was retained on that farm until subsequent inseminations and the next calving. This would have obviously allowed for better control and calculation of the relevant intervals. This survey has revealed very long intervals from calving to first service and between services. This was more common among farmers who did not keep any written records and was least among those who used computers as a data recording facility. Equally, CRs to first service and all services were lower than expected from previous information available in the field.

In conclusion, dairy farmers in Zambia share a number of hurdles to successful reproduction, some of which were identified in this study. Among these were long intervals to first AI and between services. Additionally, low CR was associated with poor record keeping and heat detection, and inadequate training of AITs and dairy farmers, particularly on reproductive health management. Despite these results, the service based on progesterone RIA presented a very reliable tool for early diagnosis of non-pregnancy and infertility. Similarly, AIDA proved to be very expedient for managing and processing data and producing very useful reports. On a number of farms, the intervals to first service and conception were being reduced through knowledge and training obtained during this study. However, better control is required not only of the cows in the study but also of their resultant offspring. Equally, farmers need to be trained on the type of records to keep for all classes of animals on the dairy farms. This would permit better implementation of remedial packages and programmes.

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