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ORIGINAL ARTICLE

Radioimmunoassay of milk progesterone as a tool for fertility control in smallholder dairy farms

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Abstract This study focused on the use of radioimmunoassay of progesterone in milk for the diagnosis of post-partum ovarian cyclicity and accurate detection of oestrus and non-pregnancy in cows in the artificial insemination (AI) programme in Bangladesh. In Investigation 1, milk samples were collected on day 0 (day of AI), day 9–13 and day 21–24 from 444 milking cows of various breeds presented for the first post-partum insemination by 413 farmers living at 182 villages/regions in Mymensingh District from 6 AI centres and sub-centres. Each cow was then examined three times after each AI until it stopped returning to oestrus. Sixty to 90 days after the last AI, the cows were examined *per rectum* to confirm the pregnancy. Milk progesterone data on day 21–24 contributed to a clear diagnosis with respect to non-pregnancy in 100% cows, indicating a possible use of this progesterone assay for identifying non-pregnant cows in AI programmes. In Investigation 2, milk progesterone was monitored two times in a month with a 10-day interval in 88 cows. The samples were taken between 10 days after calving and the first detected oestrus, followed by two more samples 10 days apart. The proportion of cows accurately detected in oestrus was 30%. Another 30% were stated to be in oestrus when they were not (false positive) and 40% were not detected when they were in oestrus (false negative). The mean intervals between calving and oestrus and between calving luteal activity were 40 to 362 days (median = 120, $n = 82$) and 34 to 398 (median = 111, $n = 64$) days, respectively. The body condition scores at calving and at the initiation of luteal activity influenced the interval between calving and luteal activity ($p < 0.05$). Cows suckled twice daily initiated luteal activity earlier than their counterparts suckled several times daily ($p < 0.05$). Determination of progesterone in milk on day 21–24 is a good means for detecting non-pregnant cows.

Keywords Cattle · Progesterone · Non-pregnancy diagnosis · Radioimmunoassay · Smallholder dairy farm

Abbreviations

AIDA	artificial insemination data analysis
BCS	body condition score
RIA	radioimmunoassay
FAO	Food and Agriculture Organization
IAEA	International Atomic Energy Agency

Introduction

Many farms in Bangladesh are so small that only one cow can be kept. Cows are tethered in a stable or

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on available grazing land. They are used for draught work as well as milk production and weaning is not controlled. These management practices promote the occurrence of post-partum anoestrus and limit behavioural manifestations of oestrus. Most *Bos indicus* cows show weak oestrus signs for a shorter duration than *Bos taurus* cows. Detection of oestrus and of the return to oestrus after unsuccessful artificial insemination (AI) is clearly difficult under such conditions and inefficiencies have been documented (Shamsuddin, 1995). Extended post-partum anoestrus, conception failure and embryonic mortality were claimed to be a major constraint limiting the reproductive efficiency in zebu and crossbred cows (Alejandrino *et al.*, 1999).

Traditionally, pregnancy diagnosis is not carried out as part of the AI programmes. Veterinary services are not always available. There is therefore is a need to introduce other methods to determine the status of cows with respect to cyclicity and pregnancy in association with AI programmes. Analysis of three milk, plasma or serum samples on day 0 (the day of AI), day 10–12 and day 22–24 after AI has been shown elsewhere to be an effective tool for monitoring the efficiency of oestrus detection, cyclicity of the cow and quality of AI services (Cai *et al.*, 2001). Usually, sampling of milk is more convenient than sampling of plasma and serum. Milk progesterone level on the day of AI retrospectively reflects accuracy of oestrus detection (Nebel *et al.*, 1987). There are reports showing high accuracy of non-pregnancy diagnosis based on milk progesterone concentrations on day 22–24 after AI in large commercial dairy farms (Kaul and Prakash, 1994). However, information on the use of such techniques in smallholder cattle farms is limited.

The aim of the present work was to use the radioimmunoassay (RIA) of progesterone in milk as a diagnostic tool to determine the status of cows in AI programmes with respect to cyclicity and pregnancy. This could lead to use of milk progesterone RIA as a tool for identifying non-pregnant cows as a service to AI programmes.

Materials and methods

The investigation included 413 farmers living in 182 villages/regions in Mymensingh District, one of the 64 administrative units of Bangladesh. Mymensingh has a medium-high type land with soil pH varying from 5.5 to 7.5 and is a part of Bangladesh usually unaffected by floods. A 50-year meteorological data summary indicated that the minimum and maximum temperatures were between 11.7 and 25.6°C and between 24.8 and 32.9°C, respectively. The humidity and rainfall varied from 67 to 87% and from 8 to 395 mm, respectively. Crop production is the main source of livelihood. Most people generate only a low income. The farmers' land totalled between 0 and 40 (median 0.6) hectares with 1–70 (median 2.0) breedable cows. Dairying is mostly of a subsistence type. Most of the cows were *Bos indicus* and others were crosses of *Bos indicus* with Holstein-Friesian and a mixture of indigenous zebus. The cows were milked 1 to 2 (median 1.0) times per day with their calves at foot. Calves survived on residual milk after the hand milking was completed. Controlled weaning was not practised; therefore, the time of spontaneous weaning was recorded. The cows were fed on rice straw, cut-and-carry grass and milling by-product as concentrate (rice polish, wheat bran and various oil cakes) with limited grazing on roadside and community land. The field work took place between August 1995 and June 1998.

Investigation 1. Milk progesterone RIA in diagnosing non-pregnancy and quality of AI services

Milking cows presented for first post-partum insemination were included in this study. For analysis of progesterone concentration, milk samples were collected in vials containing sodium azide tablets (8 mg; Merk, Darmstadt, Germany) as preservative. The day 0 (day of insemination) milk samples were collected by the AI technician immediately after AI. Usually poor farmers from the extensive farms bring the cows to the AI centres, and on commercial farms or farms owned by the rich farmers the AI operators visit the farms. If AI operators were called to the farms, they arrived within two hours. The research personnel picked up the samples within 2 days after collection. The day 9–13 and day 21–24 milk samples were collected by research personnel directly from the cow at a farm visit. The milk samples were centrifuged and skimmed milk was separated and stored at −20°C until analysed. Progesterone concentration in milk was determined using solid-phase radioimmunoassay (RIA) kits supplied by the FAO/IAEA, Vienna, Austria. The intra-assay coefficient of variation (CV) with internal quality control

(IQC) samples varied from 8.0% to 15.6% (10 assays, each with 10 replicates) and the inter-assay CVs were 16.6% and 18.4% for beginning and end IQC samples, respectively (number of assays = 10). Between day 60 and day 90 after the last recorded AI, all cows were examined *per rectum* for confirmation of pregnancy; a milk sample was always collected immediately before rectal palpation if the cow was not dry at that time. The farmers did not pay for the progesterone testing and the economic benefit obtained by the farmers was not estimated.

Investigation 2. Milk progesterone RIA in monitoring of post-partum cyclicity and accuracy of oestrus detection in cows

Eighty-eight cows on 58 farms were registered within 1 week after calving and relevant information with regard to the farm and cattle was recorded. For individual cows, information was collected on age, breed, parity, last calving date, body weight and body condition score (BCS) at calving, occurrence of oestrus, and the occurrence of any post-parturient disorders. Cows that required major assistance during parturition and/or that were diagnosed with periparturient disorders such as retained placenta, puerperal metritis, post-partum haemorrhage, prolapse of the genital tract or milk fever were not included in the investigation. We requested the farmers to report the date and signs of oestrus, but there was no discussion between the project personnel and farmers on the signs of oestrus and their relevance to conception.

The research personnel collected milk samples from individual cows twice a month, with a 10-day interval, between 10 days post partum and the first report of oestrus by the farmer. Two more samples were collected, at 10 days' interval, after the occurrence of oestrus. During milk sampling, the research personnel scored the cow for body condition (1–5 scales with 0.5 fractions), measured its body weight, asked the farmer about the occurrence of oestrus and examined the cow for the presence of any dry or fresh discharge adhering to the perineum. The milk samples were processed as in Investigation 1.

Analysis of the data

The data on milk progesterone concentrations at day 0, day 9–13 and day 21–24 were compared with the results of *per rectum* pregnancy diagnosis. Artificial insemination data application (AIDA, International Atomic Energy Agency, Vienna, Austria) was used to record and analyse the data. Progesterone data based on two samples (day 0 and day 9–13) were used to examine the efficiency of oestrus detection. Milk progesterone concentration on the day of AI was used to determine the proportions of AI performed in the luteal phase of the cow. In Investigation 2, the following model (Goodger *et al.*, 2001) was used to analyse the data:

$$\begin{aligned}\text{INT} = \mu + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 \\ + b_6x_6 + b_7x_7 + b_8x_8 + b_9x_9 + e\end{aligned}$$

where: INT = log of interval between calving and the first detected luteal activity (days); μ = general mean; x_1 = cattle rearing system (extensive vs intensive); x_2 = purpose of rearing cows (dairy vs dairy + draught); x_3 = feeding system (concentrate fed vs no concentrate fed); x_4 = breed of cow (crossbred Friesian, crossbred Sahiwal, local); x_5 = BW at calving (group 1, ≤200 kg; group 2, 201–250 kg; group 3, 251–300 kg; group 4, ≥301 kg); x_6 = BCS at calving (group 1, 1.0–2.0; group 2, 2.5; group 3, 3.0; group 4, 3.5–5.0); x_7 = BW at the first detected luteal activity (group 1, ≤200 kg; group 2, 201–250 kg; group 3, 251–300 kg; group 4, ≥301 kg); x_8 = BCS at the first detected luteal activity (groups 1, 1.0–2.0; group 2, 2.5; group 3, 3.0; group 4 3.5–5.0); x_9 = frequency of suckling (once or twice daily vs several times a day); e = error term.

The data were log-transformed to near normality.

ANOVA was used to test the effect of the accuracy of oestrus detection on the post-partum interval to oestrus (false positive cases were deleted). Unless otherwise indicated, the data are presented as median and range owing to high individual variations. Only the factors that tended ($p = 0.10$) to or significantly ($p < 0.05$) influenced the dependent variables are presented in the figure and tables.

Results

Investigation 1. Milk progesterone RIA in diagnosing non-pregnancy and quality of AI services

The cows studied were 4–18 (median 7.4) years old, their parity ranged from 1 to 12 (median 2), body

Table 1 Three-sample milk progesterone data with respect to pregnancy results

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

[a]Low, <1.0 nmol/L; [b]High, ≥3.0 nmol/L; [c]Intermediate, 1.0–3.0 nmol/L

weight was from 103 to 480 (median 196) kg, BCS was from 1 to 5 (median 3) and milk production varied from 0.3 to 16.0 (median 2) litres per day. The interpretation of progesterone data based on three samples (day 0, day 9–13 and day 21–24) with respect to pregnancy results is shown in Table 1. Milk progesterone data gave a clear interpretation in 82.5% cows (n = 360) about their pregnancy status when comparison was made with *per rectum* pregnancy diagnosis at day 60–90. None of the 75 cows with a progesterone profile of low (<1.0 nmol/L), high (≥3 nmol/L) and low on day 0, day 9–13 and day 21–24, respectively, were found pregnant at rectal palpation. Twenty-seven cows (7.5%) had a progesterone profile of low high and high on day 0, day 9–13 and day 21–24, respectively, but were not pregnant at rectal examination. Two inseminations were made in pregnant cows. All cows confirmed pregnant at rectal palpation had ≥3 nmol/L progesterone in skimmed milk at the day of pregnancy diagnosis. Cows with milk progesterone level <3 nmol/L were always non-pregnant. Eighty-one percent of cows (n = 478; including all services) had a progesterone profile of low and high on day 0 and day 9–13, respectively, indicating AI not performed at luteal phase nor during ovarian acyclicity (Table 2). Fifty-nine cows (12.3%) received AI at an incorrect time as evident from a deviant progesterone profile. Twenty-seven of 505 services (5.3%) were made when the cow had a high to intermediate level of milk progesterone.

Investigation 2. Milk progesterone RIA in monitoring of post-partum cyclicity and accuracy of oestrus detection in cows

The cows studied were 3–15 (median = 6.0) years old and of parities 1–8 (median = 3.0). At calving they weighed 160–456 (median 270) kg and BCS was 1.5–3.5 (median 2.5). The intervals from calving to the first detected oestrus and to the initiation of luteal activity were 40–362 (median 115; n = 82) and 34–398 (median 108, n = 64) days, respectively. Farmers missed detecting an oestrus (false negative) on 1–3 (median 1.0) occasions post partum. The proportion of cows accurately detected in oestrus was 30%. Another 30% were stated to be in oestrus when they were not (false positive) and 40% were not detected when they were in oestrus (indicated by one to three progesterone peaks (≥3 nmol/L) before the cow was identified as being in oestrus).

Table 2 Milk progesterone profiles of the artificially inseminated cows on the day of service and on day 9–13 with respect to the accuracy of oestrus detection

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

[a]Low =< 1.0 nmol/L;
[b]High =≥ 3.0 nmol/L
Thirty two services (6.7%) were made in cows with an intermediate level of milk progesterone (1.0–3.0 nmol/L) on day 0, on day 9–13 or on both occasions

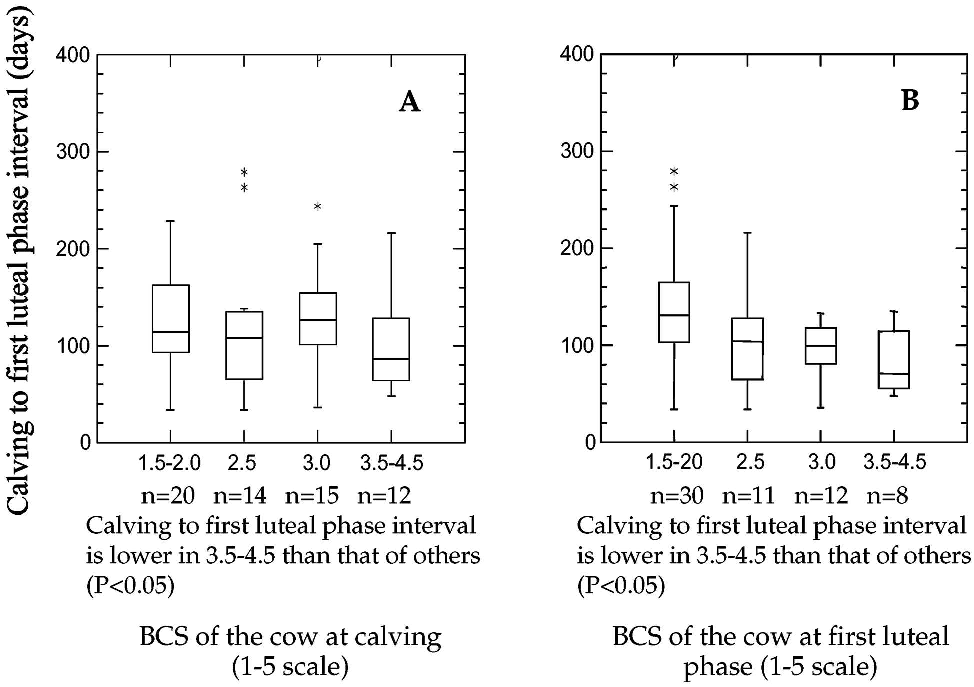

Fig. 1 Effect of body condition (BCS) at calving (A) and at first luteal phase (B) on the post-partum interval to the initiation of luteal activity

The post-partum intervals to the initiation of first luteal activity were examined in these cows. Those with BCS 3.5 or more at calving needed fewer days to initiate luteal activity than their counterparts having BCS 3.0 or less (Figure 1A, B; $p < 0.05$). Cows suckled once or twice daily required fewer days (median 95, range 34–398; $n = 28$) than the cows suckled several times a day (median 127, range 35–279; $n = 36$) ($p < 0.05$).

The median interval to first post partum oestrus was prolonged by 33.5 days due to farmers' inability to detect the oestrous cows ($p < 0.05$). The age of the cows and their parities did not influence the initiation of luteal activity ($p > 0.50$). The effects of body weight at calving, purpose of rearing cows (dairy vs dairy + draught) and breed of cow on the initiation of luteal activity were not significant ($p > 0.10$)

Discussion

Milk progesterone data based on three samples (day 0, day 9–13 and day 21–24) helped in making a clear decision about the pregnancy in 82.5% cows. If only the non-pregnancy diagnosis is concerned, milk progesterone concentration on day 21–24 (day of oestrus = day 0) will give the same results without compromising the accuracy. Milk progesterone concentration on day 0 and day 9–13 together not only identified inaccurate oestrus detection but also gave a clear indication of the cyclical status of the animals. Progesterone concentration on day 0 can only indicate whether or not AI was done in a cow with functional luteal tissues in the ovary. It was clear that cows with less than 3 nmol/L in milk on day 21–24 were always non-pregnant. Where veterinary services are not available or are too expensive, milk progesterone concentrations on day 21–24 can be used to interpret pregnancy results with 100% accuracy of non-pregnancy diagnosis (Kaul and Prakash, 1994). It is possible for a RIA laboratory to apply this approach easily in smallholder dairy farms where heat is not detected by any aids. Farmers' Knowledge of the non-pregnancy status of cows by 21–24 days would definitely help detecting the cows' return to estrus and enable them to be inseminated by 42 days without losing time until confirmation of pregnancy. Consequently, the interval from calving to conception would decrease, resulting in economic benefit to the farms even though the farmer had to wait for another cycle length to get the cow inseminated. This problem could be minimized if the non-pregnancy status of cows could be determined by 19 days and the farmer could be informed about the result of AI immediately after RIA, resulting in detection of heat by careful observation followed by AI without wasting time for another cycle. However, at the moment, owing to transportation and communication problems, it would take at least 3–4 days for the milk sample to be analysed and the result sent back to the farmer, resulting in waiting for another cycle length.

Failure to detect oestrus (false negative) and false determination of oestrus (false positive) are common problems in AI of cows in intensive farming (Smith, 1986; Dawuda *et al.*, 1989; Johnson *et al.*, 1992). False negative and false–positive categories of oestrus detection have been found by others to be as high as 30–50% (Rounsaville *et al*., 1979; Dawuda *et al*., 1989; Johnson *et al*., 1992) and 17–30% (Smith, 1986), respectively. This can be explained by the fact that in intensive farming or in small holdings having one cow, oestrus cannot be detected by primary signs such as 'standing to be mounted' as the cows are always tied up. However, the main weakness affecting the accuracy of oestrus detection is that farmers are missing or misinterpreting or are unaware of secondary signs of oestrus such as mucus discharge and swollen vulva. Nevertheless, the false positive oestrus detection was lower in Investigation 1 (12%) than that in Investigation 2 (30%). In Investigation 1, the cows were recorded at the AI centres. Firstly, this means that farmers made a positive decision about the oestrus of the cow. Secondly, the registration of cows by the AI technicians in Investigation 1 raises the question whether they made any selection in favour of good oestrus signs to prove their good performance. In Investigation 2, to avoid our frequent visits, some farmers might have made intentional false reports about oestrus. Because cows in the false-negative category are not inseminated and cows in the false-positive category are unlikely to become pregnant in that cycle, resulting in increased calving to conception interval.

Cows with good body condition at calving and thereafter initiate post-partum cyclicity earlier than those with poor body condition. This is in agreement with earlier findings (Bolanos *et al.*, 1998) and indicates the importance of good nutritional management of pregnant cows to cover the negative energy balance due to growth of the fetus and subsequent milk production (Claycomb *et al.*, 1996). A positive effect of a high nutrition plan with restricted suckling on reducing the calving to conception interval was described by Das and colleagues (1999). Early initiation of postpartum cyclicity will reduce the interval from calving to first service and to conception.

The adverse effects of the duration and frequency of suckling on the initiation of post-partum cyclicity as indicated by our results are in accordance with other reports (Williams, 1990). In a different study, we found an increased interval from calving to first service and to conception due to frequent suckling (Shamsuddin *et al.*, 2001). Controlled suckling for a restricted period favours post-partum reproduction in cows (Msanga and Bryant, 2003). The results of the present investigations do not clarify whether prolonged suckling lengthens the onset of post-partum ovarian activity or wheather the suckling continues because the cows do not dry off since they are not pregnant. However, in commercial dairy farming, controlled weaning should be practised to identify cows with an inherent tendency to remain acyclic irrespective of suckling and nutrition management.

It appears that determination of progesterone in milk on day 21–24 is a good means of making decisions on pregnancy by diagnosing the non-pregnant state with high accuracy. Farmers should have better training in oestrus detection. The nutritional condition of the cow at calving and duration and frequency of suckling are, among others, important determinants of the intervals to the initiation of ovarian activity.

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Radioimmunodosage de la progestérone du lait à titre d'outil de contrôle de la fécondité dans des fermes laitières de petits cultivateurs

Résumé – La présente étude s'est concentrée sur l'usage d'un radioimmunodosage de la progestérone dans le lait pour le diagnostic de la cyclicité ovarienne post-partum, la précision de la détection de l'œstrus et la non-gravidité chez des vaches dans le cadre d'un programme d'insémination artificielle (AI) entrepris au Bangladesh. Dans l'investigation 1, des échantillons de lait ont été recueillis au Jour 0 (jour de l'AI), aux Jours 9 à 13 et aux Jours 21 à 24 de 444 vaches allaitantes de diverses races présentées pour la première insémination post partum par 413 cultivateurs vivant dans 182 villages/régions du district de Mymensingh à 6 centres et sous-centres de AI. Chaque vache a ensuite été examinée trois fois après chaque AI jusqu'à ce qu'elle cesse d'être fécondable. Soixante à 90 jours après le dernier AI, les vaches ont été examinées par le rectum pour confirmer la gravidité. Les données de la progestérone du lait aux Jours 21 à 24 ont contribué à un diagnostic irréfutable de non-gravidité chez 100% des vaches, indiquant la possibilité d'utiliser ce dosage de la progestérone pour l'identification des vaches non gravides dans le cadre des programmes AI. Dans l'investigation 2, la progestérone du lait a été contrôlée deux fois en un mois à 10 jours d'intervalle chez 88 vaches. Les échantillons ont été prélevés entre 10 jours après le vêlage et le premier oestrus détecté, suivis de deux échantillons de plus à 10 jours d'intervalle. La proportion des vaches détectées avec précision en oestrus a été de 30%. Une autre proportion de 30% des vaches a été déclarée être en oestrus alors qu'elles ne l'étaient pas (faux positifs) et une proportion de 40% des vaches n'ont pas été détectées alors qu'elles étaient en oestrus (faux négatifs). Les intervalles entre le vêlage et l'œstrus et l'activité lutéale ont été de 40 à 362 (valeur médiane = 120, $n = 82$) et 34 à 398 (valeur médiane = 111, $n = 64$) jours, respectivement. Les scores de la condition corporelle (BCS) au vêlage et au commencement de l'activité lutéale ont influencé l'intervalle entre le vêlage et l'activité lutéale ($p < 0.05$). Les vaches tétées deux fois par jour ont commencé une activité lutéale plus tôt que leurs homologues tétées plusieurs fois par jour ($p < 0.05$). La détermination de la progestérone dans le lait aux Jours 21 à 24 est un bon moyen de détection des vaches non gravides.

Radioinmunoanálisis de progesterona en leche como herramienta para el control de la fertilidad en pequeñas granjas lecheras

Resumen – El presente estudio se centró en la utilización de radioinmunoanálisis de progesterona en leche para el diagnóstico de la ciclicidad ovárica post-parto, la precisión de la detección del estro y la ausencia de preñez en vacas en el programa de inseminación artificial (IA) de Bangladesh. En la investigación 1, se recogieron muestras de leche en el día 0 (día de IA), días 9–13 y días 21–24 a partir de 444 vacas de ordeño de diferentes razas presentadas para la primera inseminación post-parto a 6 centros y subcentros de IA, por 413 granjeros que vivían en 182 pueblos/regiones del Distrito de Mymensingh. Cada vaca era luego examinada tres veces después de cada IA hasta que paraba de regresar al estro. Se examinó el recto de las vacas, a los 60–90 días después de la última IA, para confirmar el embarazo. Los datos de la progesterona en leche en los días 21–24 contribuían a un claro diagnóstico con respecto a la no preñez en el 100% de las vacas, indicando con ello una posible utilización de este análisis de progesterona para identificar a las vacas no preñadas en los programas de IA. En la investigación 2, se monitorizó la progesterona de la leche, dos veces al mes con un intervalo de 10 días, en 88 vacas. Las muestras fueron tomadas entre 10 días después del parto y el primer estro detectado, seguidas por dos muestras más con 10 días de diferencia. La proporción de vacas a las que se les detectó con precisión el estro fue de un 30%. Otro 30% fue declarado estar en estro cuando en realidad no estaban (falso positivo) y a un 40% no se les detectó cuando se hallaban en estro (falso negativo). Los intervalos entre partos y estros, y la actividad luteínica fueron de 40 a 362 (media = 120, $n = 82$) y 34 a 398 (media = 111, $n = 64$) días, respectivamente. La puntuación de la condición corporal (PCC) en el parto y al inicio de la actividad luteínica influía en el intervalo entre partos y actividad luteínica ($p < 0.05$). Las vacas que daban de mamar dos veces al día iniciaban la actividad luteínica más pronto que sus compañeras que daban de mamar varias veces al día ($p < 0.05$). La determinación de la progesterona en la leche en los días 21–24 es un buen medio para detectar a las vacas no preñadas.