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

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Abstract

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

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

1. INTRODUCTION

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

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

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

The objectives of the present studies were:

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

2. MATERIALS AND METHODS

2.1. Surveys

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

2.2. 1995 survey

2.2.1. Farms, animals and management

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

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

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

2.3. 1996 survey

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

2.4. Milk sampling and progesterone analysis

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

2.5. Interoestrus intervals

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

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

2.6. Statistical analysis

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

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

where:

INT = Interval to first service or to conception
a = the ith effect of region
b = the effect of farm (A, B, C, D and E);
c = the kth effect of parity (1 and 2);
d = the lth effect of calving type (1, 2, 3 and 4);
e = the mth effect of BW (350, 450, 500, 550, 600, 650 kg);
f = the fth effect of BCS at Calving (1, 2, 3);
g = the oth effect of month of calving (1 to 7);
h = the pth effect of month of breeding (5 to 12);
i = the qth effect of BW at service (450, 500, 550, 600, 650 kg);
j = the rth effect of BCS at service (1, 2, 3);
k = the sth effect of milk production at service (5, 10, 15, 20 25, 30 L);
$\varepsilon_{ijklmnopqrs}$ = aleatory error;

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

2.7. Factors affecting reproductive efficiency

2.7.1. Farms, animals and management

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

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

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

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

2.7.2. Methodology

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

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

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

2.9. Statistical analysis

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

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

where:

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

μ: Overall mean

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

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

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

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

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

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

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

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

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

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

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

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

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

2.11. Statistical analysis

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

3. RESULTS

3.1. Survey

3.1.1. Overall reproductive performance

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

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

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

[1]: n: number of cows

^{a,b,c}: Different letters within columns differ (P <.05)

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

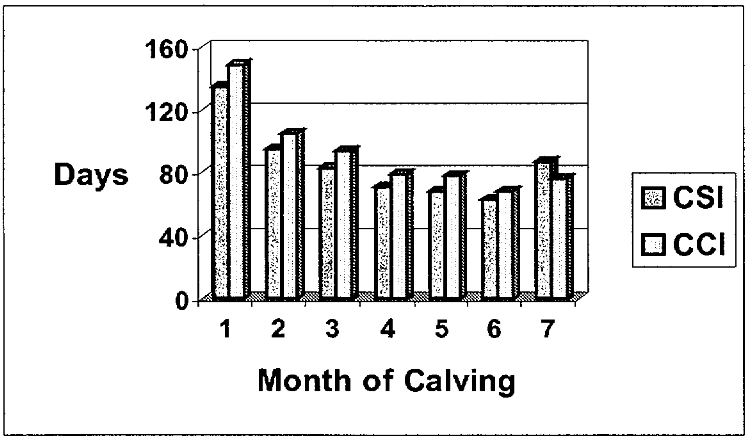

FIG. 1. Average intervals from calving to first service (CSI) and to conception (CCI) according to month of calving.

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

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

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

$^{a, b}$: Different letters within columns by year and by parameter differ ($P < 0.01$)

$^{c, d}$: Different letters within columns by year and by parameter differ ($P < 0.001$)

3.1.2. Evaluation of AI services by milk progesterone

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

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

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

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

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

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

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

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

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

3.1.3. Precision of oestrus detection

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

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

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

^{a,b}: (Chi Square = 72.6, $P < 0.05$)

3.1.4. Pregnancy estimation (1996)

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

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

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

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

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

^{a}: $P > 0.1$

3.1.6. Interoestrus intervals

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

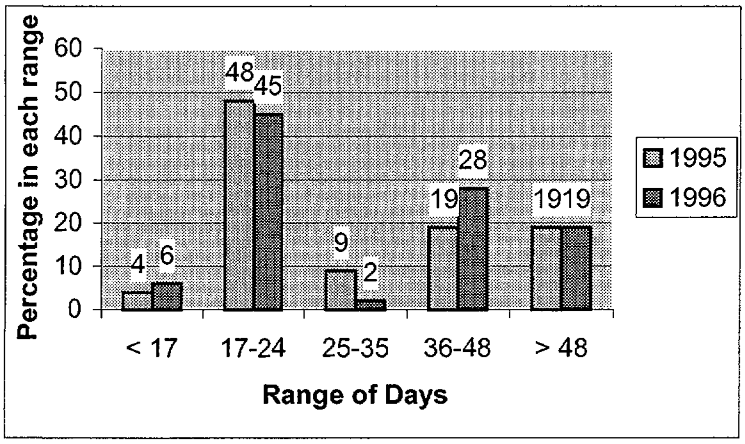

FIG. 2. Percentage of oestrous cycles of different duration

3.2. Factors affecting reproductive efficiency

3.2.1. Incidence of postpartum anoestrus

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

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

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

$^{a, b, c}$: P <0.05; $^{d, e}$: P <0.01

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

3.2.2. Ovarian activity

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

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

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

$^{a, b, c}$: Different letters for each variable differ, P <0.05

3.2.3. *Factors affecting oestrus detection efficiency*

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

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

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

$^{a, b}$: Different letters within columns (P < 0.05); $^{c, d}$: Different letters within rows (P <0.05)

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

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

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

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

3.3. Response to oestrus synchronisation

The percentage of cows with luteal levels of milk progesterone at beginning of treatment (day 0) was 57.7%, at PGF injection (day 7) was 69.3% and at 24 hours after PGF injection (day 8) was 8.7%. The number of cows inseminated within 30 days after PGF injection is presented in Table XI.

TABLE X. INTERVAL FROM THE BEGINNING OF THE BREEDING PERIOD TO THE FIRST SERVICE BETWEEN FARMS, DAYS POSTPARTUM AND PARITY (DAYS, MEAN ± SEM)

PARAMETER	CATEGORY	DAYS
FARM	A	32.8 ± 2.3^{a}
	B	16.1 ± 2.3^{b}
	C	36.1 ± 2.0^{a}
DPP	40–60	31.7 ± 2.3^{a}
	61–90	30.4 ± 2.1^{a}
	>90	23.3 ± 2.1^{b}
PARITY	1	30.1 ± 2.2^{a}
	2+	25.9 ± 1.5^{a}
OVERALL		27.4

[a, b]: Different letters within rows differ ($P < 0.05$)

TABLE XI. NUMBER OF COWS TREATED, PERCENTAGE DETECTED IN HEAT AND INSEMINATED AND INTERVAL FROM END OF TREATMENT TO INSEMINATION IN EACH FARM

PARAMETER	FARM A	FARM B
NUMBER OF COWS	171	243
% INSEMINATED[1]	$71\ \%^{a}$	$80\ \%^{a}$
INTERVAL PGF-AI[2]	13.2 ± 1.5^{b}	6.1 ± 0.6^{a}

[1] Cows inseminated within 30 days after treatment

[2]: INTERVAL PGF-AI = Prostaglandin injection to AI (days, mean ± SEM)

[a,b]: Different letters within rows differ ($P < 0.05$)

Although not statistically different, a greater percentage of cows were detected in heat and inseminated in farm B. A statistical difference was found in the interval from end of treatment to AI, with 13.2 ±1.5 days for farm A and 6.1 ± 0.6 days for farm B ($P < 0.05$).

When the interval form PGF injection to AI was divided in three periods (Table XII), the percentage of cows inseminated within 5 days after treatment was significantly lower in farm A with a conventional heat detection system (59.8%), compared to farm B where oestrus was detected three times per day (77.7%, $P < 0.07$). While no differences were found in the percentage of cows inseminated between days 5 and 22 after treatment ($P > 0.05$), a greater percentage of cows were inseminated between day 22 and day 30 in farm A (27.9%) than in farm B (7.8%) ($P < 0.01$).

TABLE XII. PERCENTAGE OF COWS INSEMINATED IN THREE PERIODS AFTER TREATMENT

INTERVAL PGF-AI[1]	FARM A	FARM B
<5 days	$59.8\ \%^{a}$	$77.7\ \%^{b}$
	(73/122)	(150/193)
5–22 days	$12.3\ \%^{c}$	$14.5\ \%^{c}$
	(15/122)	(28/193)
>22 days	$27.9\ \%^{d}$	$7.8\ \%^{e}$
	(34/122)	(15/193)

[1]: INTERVAL PGF-AI: Interval from Treatment to AI

[a, b]: $P < 0.07$, [c]: $P > 0.1$ [d, e]: $P < 0.01$ (different letter within rows)

Figure 3 shows the percentage of cows inseminated per day. More than 40% of the cows were bred within 2 days after treatment.

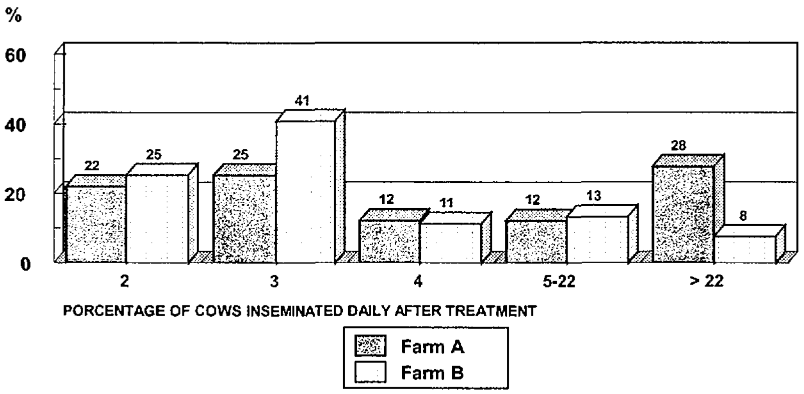

FIG. 3. Percentage of cows inseminated daily after treatment on each farm.

3.4. Precision of oestrus detection

From a total of 467 milk samples analysed from 315 cows synchronised and 152 bred at natural oestrus, only 5.7% had progesterone values higher than 1 nmol/L and of these 2.3% had values above 3 nmol/L. No difference was found between farms or cows bred at a synchronised or natural heat.

First service conception rate in farm A was 56.9% with a difference between synchronised (65.6%) and controls (48.1%) (P = 0.11), while in farm B fertility was very low (30.5%) without differences between synchronised (30.9%) or controls (30.1%). These results are presented in Table XIII.

TABLE XIII. CONCEPTION RATE BY FARM FOR COWS SYNCHRONISED OR INSEMINATED AT NATURAL OESTRUS

GROUP	FARM A	FARM B
SYNCHRONISED	65.6^{a}	30.9^{a}
CONTROLS	48.1^{b}	30.1^{a}
TOTAL	56.9	30.5

^{a,b}: Different letters within columns differ (Chi Square$_1$, P = 0.11)

4. DISCUSSION

4.1. Survey

4.1.1. Reproductive parameters and factors that affect them

Longer intervals from calving to first service (CSI) and to conception (CCI) for region 1 in 1995 were due to a management factor, as spring breeding season in 1994 was eliminated. Thus, cows that calved in winter/spring of 1994 had a longer voluntary waiting period and their first breeding was in May of 1995. There were differences in regions 2 and 3 in the CSI and CCI. Farms of region 3 were small dairies within an extensive region and breeding period was seasonal (winter and spring). This longer interval was due to cows that did not become pregnant in one season and are not bred again until the next season. This also resulted in the lower overall pregnancy rate.

In both years, there was a confounding effect between the month of calving and the interval to the first service and, thus, to conception. This was due to cows with calvings in January and February that had a longer voluntary waiting period because the breeding season started in May. Another confounding effect occurred in Region 3 (1995). This region had two short breeding periods and some cows calving in winter/spring (months July through September) were not bred in the following spring breeding season (October to December), probably due to prolonged postpartum anoestrous. A significant interaction between month of calving and region was detected for these intervals (P <0.001).

Reproductive parameters in both surveys for region 2 were similar, suggesting that year effect was not so marked in farms where grazing was supplemented with hay, silage and concentrates, so feeding did not totally rely on climatic factors. These parameters were similar to those reported for temperate climates [19, 20, 21] or for dry tropics [22]. Mean interval from calving to first service of 106 days (1995) and 102 days (1996) were considerably longer than the goal of 65 days proposed by Morrow [23]. One of the reasons of this longer interval was a prolonged voluntary waiting period due to the seasonal breeding system, a similar effect as reported in New Zealand systems [24]. Figure 1 illustrates this effect and, because the breeding season started on May 20th, cows that calved in January had an interval to first service of 155 days, as opposed to those calving in April that had an interval of 89 days. However with a voluntary waiting period of 40 days the interval never was shorter than 70 days and this can be caused by a prolonged postpartum anoestrous or a poor heat detection, which the methodology used in the survey was not able to differentiate.

Interval to first service was also affected by parity, agreeing with previous reports [25, 26]. Body weight and body condition at calving also affected this parameter, similar to findings by Langley and Sherrington [27]. These authors found that cows with body condition of less than 2.5 at calving had an interval to service of 80 days as opposed to 47 days for animals calving with a body condition greater than 2.5.

4.2. Evaluation of AI services by milk progesterone

Milk progesterone determination is an important diagnostic tool in large herds and has been widely used [16, 28, 29]. In these surveys, cows incorrectly detected in heat ranged between 13.5% (1995) and 11.1% (1996) a greater error than reported by authors that describe percentages lower than 10% [29, 30]. However, errors around 20% have been more commonly found [31, 32, 29]. The error was consistent in different breedings but an important variation among farms was detected as also reported previously [29]. According to Zarco [10] one of the main causes of cows incorrectly detected in heat is the human factor.

Conception rate in cows bred with high progesterone values is zero [28, 33]; however in the 1996 survey, from 206 cows inseminated with progesterone values greater than 1 nmol/L, 44 (21.4%) were reported pregnant from that breeding. From these animals, 71 had progesterone values between 1 and 3 nmol/L and 21 conceived (29.6%). The criteria to decide whether a cow was in oestrus or not was receptivity to be mounted and probably some cows in prooestrus or early metoestrus were reported in heat and inseminated with the results mentioned above.

4.2.1. Pregnancy estimation by milk progesterone and early embryonic death

Progesterone levels obtained on days 22 to 25 after breeding have 100% accuracy in diagnosing non-pregnant cows, but precision of the positive pregnancy diagnosis is around 80% [28, 33, 34, 35]. In the 1996 survey, pregnancy estimation by milk progesterone at day 23 after breeding was only 70.4%, lower than some reports, but closer to the results of Rajamahendran and co-workers [30]. According to these authors, early embryonic mortality is a cause more important than oestrus detection or reproductive failure. Forar and co-workers [24] found an embryonic mortality rate of 10.8% with a period of greater risk between days 31 and 55 of gestation. In this study the mean interval to next heat for cows diagnosed as possibly pregnant by milk progesterone was 57 days, which agrees with the above study.

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

Oestrus detection efficiency was 37.5%, similar to previous reports ranging from 38-43% [20, 21, 22], but lower than some reports of 52% [19] and 74% [36]. With a conception rate of 42% and an oestrus detection efficiency of 37.5%, pregnancy rate was of only 16%. In dairy systems with restricted seasonal breeding periods, this low PR seriously compromises the goal of a 12-month calving interval and partially explains the prolonged period to the first service.

4.2.3. Interoestrus intervals

The analysis of the interoestrus intervals is another way of measuring heat detection efficiency [19, 9]. Evaluated by this method, the efficiency was 58%, which is higher than the 37.5% calculated previously by considering only the first service. A possible cause of this difference is that when only the efficiency of the first breeding is considered, cows in anoestrus were included, which could not be identified under the methodology of the study. According to Esslemont [19] with a good oestrus detection efficiency the ratio between normal interoestrus intervals (17 to 24 days) and abnormal intervals should be 7:1, much higher than the 2.2:1 ratio found in this study. Expressed in another way, from 100 interoestrus intervals, 74% should be within the normal range, higher than the 45% reported here. This analysis confirms the poor oestrus detection efficiency, although it is possible that other factors such as high embryonic mortality due to pathological causes had affected this results.

4.3. Factors affecting reproductive efficiency

4.3.1. Incidence of postpartum anoestrus

Postpartum anoestrus determined by milk progesterone was 12.5% and the population with higher incidence was first-calf heifers (82% of this 12.5%). This is greater than the 6% reported by Bloomfeld and co-workers [37] in cows on pasture in the United Kingdom. The difference was most probably due to a better nutrition in those animals. Similar situations have been described in New Zealand [38, 39] and indirectly relate to lower pasture availability with a higher stocking rate, leading to prolonged postpartum anoestrous periods. According to Macmillan and co-workers [40], non-cycling cows represent the most important infertility problem in New Zealand dairy herds and this condition is a reflection of insufficient energy in the diet after calving and during the breeding season.

Furthermore, in the present study 52% of the ovulations determined by milk progesterone were never observed, similar to previous reports [21, 41]. Also, 8.5% of the cows with normal ovarian activity as determined by milk progesterone were never detected in heat during the 80 days of the experimental period. There was a strong farm effect on this parameter. Adding the percentage of first-calf heifers in anoestrus to those not detected in heat, 37% of the animals in this category were not bred during the season. A similar problem is described in New Zealand [40] in cows in pasture and Fagan and co-workers [42] concluded that the main factor affecting reproductive efficiency was heat detection, but especially in first-calf heifers that are the future of the enterprise.

4.3.2. Factors affecting oestrus detection efficiency (HDE)

Oestrus detection efficiency during the first period of 21 days was 46.7%, greater than the 37.5% found in the survey where all cows were considered independently of their ovarian activity. Differences among periods were not significant. The important differences between farms can be explained by the human factor, as there was only one person in each farm doing the heat detection and this was the same during the three periods. It is interesting to see that the three farms show different situations. In farm A, oestrus detection efficiency was very poor during the first period (28.8%), improving in the second (50 %) and in the third (81.8%). Apparently, as the number of cows to be bred decreased, the efficiency of the detection increased, although this is in contradiction with a previous study [14] which found that a greater number of cows in heat increases sexual activity and thus indirectly increases the possibility of detecting them. Possibly this discrepancy is due to the mechanics of oestrus detection in this system, as it is done when the cows are taken to the milking parlour and, when many cows are in heat at the same time, human failure in identifying and/or recording them all could arise. In farm B, on the contrary, there was high oestrus detection efficiency in the first period (76.4%), decreasing in the second (47.6%). Although there is no firm basis to demonstrate it according to the experimental design, it could be assumed that with good heat detection, all those cows with clear oestrus signs should be detected at their first oestrus. Those ones with weaker symptoms remained for the second period, making the task of detecting them more difficult. Farm C was consistently inefficient in the three periods, agreeing with King and co-workers [43] who concluded that a high percentage of the difficulties in oestrus detection are due to human error or farm management systems rather than a problem with individual animals. The only other factor affecting oestrus detection was parity, again in concordance with previous reports [15].

4.3.3. Interval from the beginning of the breeding period to the first service

If the oestrus detection efficiency were 100% and all the animals were detected during the first 21 days, considering that 4-5% of the cycling animals would be in heat on any one day, the mean interval from the beginning of the breeding season to the first service would be 12 to13 days. Evaluation of this parameter is very useful in systems with seasonal breedings [4, 44] as it allows relating reproductive efficiency with parameters such as intervals from calving to first service, conception and next calving. The interval found in this study was 27.4 days, longer than the intervals of 13 and 15 days reported previously [44, 38], where oestrus detection efficiency was 90%. There was also a marked farm effect on this parameter, which is related to the oestrus detection efficiency of each farm. There were no differences in parity but cows with more than 90 DPP had an interval significantly shorter, again related to a greater heat detection rate in these cows.

In systems with seasonal breeding periods, the prolongation of this interval seriously compromises reproductive efficiency, as it limits the possibilities of having a cow pregnant by the end of the period. Poor oestrus detection rates lengthen the interval to first service and, when conception rate is also low (40%), overall pregnancy by the end of the period would be greatly affected [45].

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

4.4.1. Progesterone levels

According to progesterone levels at day 0 (beginning of treatment) less than 60% of the cows had luteal values at that time, which is lower than the 71% expected when considering that the dioestrous phase lasts on average 15 days [46]. This difference was probably due to cows in anoestrous that were incorrectly diagnosed as cycling by rectal palpation. Progesterone levels at the time of PGF injection (day 7) were also lower than those reported in studies with cows with ovarian activity determined by rectal palpation at treatment [47]. It is possible then, that a percentage of cows were in anoestrus at the beginning of the treatment. Less than 10% of the cows maintained high milk progesterone levels 24 hours after PGF, a similar response as reported by Twagiamungu and co-workers [48], but lower than the findings of Moreno and co-workers [49] where 100% of the cows regressed the CL after treatment. In the present trial, those animals were not detected in heat within 5 days after treatment as should be expected.

4.4.2. Response to the treatment

Results in Figure 2 show that in the farm with two heat detection periods the response to the treatment was less clustered than in farm B with three detection periods. In the farm with three detection periods a higher percentage of cows were inseminated following treatment. Previous studies have shown that with four periods of oestrus detection 75% of the cows in heat can be detected [50], and with 12 daily observations it is possible to detect 100% of the cows in oestrus [12]. Interval from treatment to insemination was significantly shorter in the farm with more periods of observation. According to previous reports [28] the response to the synchronisation occurs within the 5 following days, with 70-80% of the cows in heat [48]. The lower percentage of cows detected in heat with the twice a day system was due to a greater percentage of undetected heats. Animals that did not responded to the treatment and maintained high milk progesterone levels 24 hours after prostaglandin injection were bred between a 5 and 22 days period, and the percentage was similar in both farms. The greater number of cows detected in heat between days 22 and 30 in farm A corresponded to those responding to the synchronisation treatment but not observed in heat in the first 5 days and were consequently inseminated at the following oestrus.

4.4.3. Precision of oestrus detection and fertility after treatment

The percentage of cows with luteal levels of progesterone when reported in heat were very low (5.7%) and there was no difference in cows with synchronised or natural heats. According to Fogwell and co-workers [52] synchronisation protocols that include an exogenous source of progesterone control oestrus expression better and could be the reason for this low percentage.

In farm A, that had a "normal" conception rate, there was a slight increase in fertility in the group synchronised with a progestin implant. However, larger number of animals would de needed to be able to assess an increase of fertility with this treatment.

5. CONCLUSIONS

The main factor affecting AI services in seasonal breeding programs in Uruguay is poor oestrus detection efficiency. This is mainly due to a human factor, as the greatest differences were detected between farms. The consequence of this is a prolonged interval to first service and to conception (days open). Other factors affecting reproductive efficiency were body weight and body condition at calving and parity. Precision of oestrus detection was not a major problem, although farm differences were also detected in this parameter.

In well-managed dairy herds in Uruguay, postpartum anoestrus is not a major reproductive constraint, although the first-calf heifers are the most susceptible category. Failure to detect cows in oestrus is the major factor affecting reproductive efficiency and this is caused by a human factor, as the greater variation in oestrus detection rate is among farms. In seasonal breeding programs, this oestrus detection failure seriously compromises the reproductive efficiency and the farm management, as more cows would be culled due to reproductive problems.

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