POSTPARTUM OVARIAN ACTIVITY AND ANOVULATORY ESTRUS IN PRIMIPAROUS CROSSBRED COWS IN THE VENEZUELAN TROPICS

Actividad ovárica postparto y celos anovulatorios en vacas primíparas mestizas en el trópico venezolano

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ABSTRACT

Progesterone (P4) profiles were studied in 61 crossbred primiparous postpartum dairy cows with normal parturition. Cows were located in a dry tropical environment (29.5°C and 1,048 mm annual precipitation), grazing in Guinea grass (*Panicum maximum*) and Survenola grass (*Digitaria xumfolozi*) supplemented with concentrate feeding according to milk production level, and body condition. The first postpartum estrus (FPE) was detected by visual observation twice a day. Rectal examination was performed once a week. Milk samples were taken twice a week beginning seven days postpartum. P4 levels in skim milk were determined by radioimmunoassay, as an indicator of ovarian activity (≥ 5 ng/ml). A first progesterone secretion (FPS) prior to the First Postpartum Estrus (FPE) was observed in 50.8% of the cows at 42 ± 27 postpartum days (ppd). This FPS was ≤ 10 days in the 87.1% of the cases and 11-17 days in the rest of the animals (12.9%). The FPE was detected at 56 ± 32 ppd; 10.9% of the FPE were anovulatory estrus (AE). Cows with AE were more frequently observed before 45 ppd. Only 38.2% of the FPE was detected prior to 45 ppd; the FPE was observed whitin 60 postpartum days in the 68.8% of the cows. Six cows (9.8%) were in luteal phase while 1.64% had persistent high P4 values (> 24 days) during the calving to FPE interval. An agreement of 44.64% was observed between P4 levels ≥ 0.5 ng/ml and a detectable corpus luteum, and 84.74% after FPE. The FPE and P4 level ≥ 0.5 ng/ml interval was 7.4 ± 3.6 days.

Key words: Progesterone, postpartum ovarian activity, crossbred cows, anovulatory estrus.

RESUMEN

Los perfiles de progesterona (P4) postparto fueron estudiados en 61 vacas mestizas lecheras con partos normales. Las mismas se encontraban en un clima de Bosque Seco Tropical (*29,5°C y 1.048 mm de precipitación anual*), *pastoreaban* pasto Guinea (*Panicum maximum*) y Survenola (*Digitaria xumfolozi*). Fueron suplementadas con alimento concentrado de acuerdo al nivel de producción y la condición corporal. Para la detección de celo y determinación del inicio de la actividad ovárica, se realizaron observaciones dos veces al día, examen transrectal (1 vez/semana) y determinación de progesterona *en leche (2 veces/semana) a partir del séptimo día postparto*. Niveles de progesterona fueron determinados por radioinmunoensayo, considerando $\geq 0,5$ ng/ml indicador de actividad luteal. Una primera secreción de P4 (PSP4) previa al Primer Celo Postparto (PCP) fue observado en el 50,8% de las vacas a los 42 ± 27 días postparto (dpp). Esta PSP4 ≤ 10 días ocurrió en el 87,1% de los casos y de 11-17 d en el resto de *los animales (12,2%)*. El PCP fue detectado a los 56 ± 32 dpp. El 10.9% de los PCP fueron anovulatorios (CA). Las vacas con CA fueron observadas más frecuentemente antes de los 45 dpp. El 38,2% de los PCP fueron detectadas antes de los

45 dpp. El 68,8% de las vacas fueron detectadas en celo dentro de los 60 dpp. Celos en fase luteal fueron detectados en el 9,8% de las vacas y 1,64% exhibieron niveles persistentes de P4 (> 24 días) durante el intervalo parto-PCP. Una concordancia del 44,6% fue observada entre un nivel de P4 ≥ 0,5 ng/ml y la detectable presencia de un cuerpo lúteo, esta concordancia fue del 84,74% después del PCP. El intervalo entre el PCP y la detección de nivel de P4 ≥ 0,5 ng/ml fue de 7,4 ± 3,6 días.

Palabras claves: Progesterona, actividad ovárica, postparto, vacas mestizas, celo anovulatorio.

INTRODUCTION

The periparturient period and the onset of the postpartum ovarian activity have been recognized as critical events in the reproduction of the cows [17]. The calving interval is directly affected by the open period, which is prolonged in crossbred first calving heifers of the Maracaibo Lake basin Venezuela [7]. The interaction of three factors: 1) resumption of the postpartum ovarian activity; 2) estrus detection efficiency, and 3) conception rate, are responsible for the calving to conception interval. Cows have a well-known normal physiological postpartum anestrous period [16]. Lactation and suckling have been identified as ovulation inhibitors [9, 23]. Furthemore an estrus ovulation dissociation during the postpartum period has been also widely reported [2, 5, 11, 15, 17, 19, 22, 24, 25]. Crossbred primiparous cows have been reported to attain normal fertility after 30 days postpartum [21]. The objectives of this study were to determine the initiation of postpartum ovarian activity of crossbred nonsuckled dairy cows, by measuring progesterone levels; and to evaluate the incidence of estrus without ovulation during the early postpartum.

MATERIALS AND METHODS

The research was carried out at the University dairy farm, located at 10° 15' latitude north 71° 25' longitude west and 100 m above sea level. The region was classified as a dry tropical area, with a mean temperature of 29.5° and an annual rainfall of 1,048 ± 180 mm. Sixty one primiparous crossbred (*Bos taurus: bos indicus: about 50:50%*) cows were studied. All cows had normal delivery and their calves were removed 72h after parturition. The cows were subsequently milked twice daily (from 4 to 6 AM and from 4 to 6 PM). They grazed on Guinea (*Panicum maximun*) and Survenola (*Digitaria xunfolozi*) grass, except during the dry months of the year when they were given hay from the above mentioned grasses. During the milking period concencentrate feeding 20% of crude protein, 2% ether extract, 10% of crude fibre, calcium 0.7% and phosphorus 0.6%; the TDN was 73-74% and energy supplementation with the supplement, was offered according to the milk pro-

duction levels as follows: Cows giving 5.9, 6 to 9.9, 10 to 13.9, 14 to 19.9, 18 to 21.9 and > 22 kg of milk per day received no supplement or 2, 4, 6, 8 and 10 kg of supplement, respectively. Estrous detection was twice daily until the first postpartum estrus, with the exception of those cows that showed estrus prior to 20 days postpartum. These cows were kept under observation until second estrus. At calving, body condition scores and weights of the cows were obtained around the third postpartum day and then weighing was carried out monthly, until the first postpartum estrus.

Visual observation twice a day using a teaser bull, fitted with a chin ball marker, were used for estrus detection. Ovarian activity and uterine involution were monitored weekly by transrectal palpation beginning and until the first detected estrus. Progesterone (P4) was measured on two milk samples from each cow, twice a week (Monday and Friday), and taken in test tubes previously treated with sodium azide in order to obtain a 0.1% final concentration. The whole milk sample was centrifuged at 3,000 RPM for ten minutes and the skin milk was kept frozen at -20°C until laboratory processing. Samples were taken from the right front quarter at the beginning of the first morning milking. Sampling was continued for minimum of three samples after onset of the first postpartum estrus. Defatted milk samples were assayed in duplicate for progesterone by radioimmunoassay using the RIA/kit, Coat-A-Count Progesterone (Diagnostic Products Corporation) validated by Plaizier, [18]. Each sample was processed twice according to instructions. Skim milk peogesterone concentrations at or above 0.5 ng/ml were considered to be an indicator of luteal activity [2, 13]. Intra and inter-assay variation coefficients were 8.9 and 13.2 respectively, as determined [20]. In order to establish the progesterone levels between samples, a modification of Blommfields rule was used as presented in TABLE I.

Having chosen the threshold concentration low (L) and high (H) progesterone, a progesterone profile can be represented by a sequence of single character codes. A sequence of codes would be written thus L... L.. H... H.. L... H where L represents a sample with a low P4 concentration (≤ 0.5 ng/ml), H a sample with a high P4 concentration (> 0.5 ng/ml), and a dot represents a day when no samples were taken. No codes were assigned to days before the first milk sample was taken.

The sequences were completed by replacing each dot with L or H according to the arbitrary rule given in TABLE I. The above pattern yields the following sequence: LLLLLLLLHHHHHHHHLLLLH

Using table 1 and considering the duration of progesterone levels ≥ 0.5 ng/ml before the first postpartum estrus, luteal activity (cicles) of all cows was classified as ultrashort < 4 days, short 5-10 days, normal 11-17 days and long 18-24 days.

Resumption of ovarian activity was determined by detection of the first progesterone secretion ≥ 0.5 ng/ml previous to the first detected estrus and/or visual observation of the cow

TABLE I

RULE FOR COMPLETING THE SEQUENCES OF CODED PROGESTERONE CONCENTRATION

Preceding sample CODED	Following sample CODED	
	L	H
L	L	L
H	H	H

TABLE II

,LUTEAL ACTIVITY LENGHT PREVIOUS TO THE FIRST POSTPARTUM ESTRUS IN CROSSBRED PRIMIPAROUS COWS IN THE TROPICS

Reproductive events	n	%
First luteal activity (lenght)*		
- Ultrashort (< 4 d)	14	45.16
- Short (5-10 d)	13	41.54
- Normal (11-17 d)	4	13.30
- Long (18-24 d)		
Total	31	100
First postpartum estrus		
- Ovulatory	49	89.1
- Anovulatory	6	10.9
Total	54	100

* Lenght of luteal activity (progesterone ≥ 0.5 ng/ml)

accepting to be mounted by another cow or the teaser bull. Anovulatory estrus was considered when three skim milk samples having progesterone levels < 0.5 ng/ml after estrus were detected.

Acyclic cows were considered to be those females not having cyclic progesterone changes (P4 ≤ 0.5 ng/ml) even when showing estrus behavior. Cyclic cows on the other hand were those exhibiting changes in the progesterone profile of the least one cycle before the first 60 days postpartum.

RESULTS

Weight at calving and at first estrus was 388 ± 5.12 kg and 396 ± 5.2 kg respectively. The first postpartum estrus was detected al 56 ± 32 ppd.

A progesterone secretion previous to the first detected estrus was observed in 31 animals (50.8%) at 42 ± 27 ppd, TABLE II. Duration of the first luteal activity was shorter or equal

TABLE III

FIRST POSTPARTUM ESTRUS AND PROGESTERONE LEVEL ACCORDING TO POSTPARTUM DAYS IN PRIMIPAROUS CROSSBRED COWS

First postpartum estrus	45 ≤ ppd		> 45 ppd	
	n	%	n	%
Ovulation	17	34.6	32	65.4
Anovulation	4	66.7	2	33.33
Total	21	38.2	34	61.8

ppd = postpartum days. n = number of observation.
chi-square ns (p> 0.05)

to ten days in 86.7%, and from 11 to 17 days in 13.3 of the cases. Luteal activity longer than 18 days was not observed. First postpartum estrus was ovulatory in 89.1% and anovulatory in 10.9%.

The first postpartum estrus (FPE) classified according to the number of days postpartum when the FPE was detected is shown in TABLE III. The results indicated that 66.7% of the anovulatory estrus activity were observed before 45 days postpartum. In addition 38.2% of the total estrus activity was exhibited during the same period. The results also showed that after 45 days postpartum estrus was ovulatory in 65.4% of the cases; the chi-square analysis indicates a non-significant difference (P>0.05) showing a non existent dependence between ovulatory and/or anovulatory estrus and the postpartum days. Before 60 days postpartum 68.8% of the cows were detected in estrus and 67.2% of them showed one luteal cycle during the same period. Prolonged postpartum anestrus was found in 31.1% of the total cows and 8.2% acyclic cows representing 27% of anestrus animals. Cows detected in estrus with high levels of progesterone, (luteal phase), were found 9.8% of the sample time, while a low porcentage (1.6%) showed high levels of progesterone (≥ 0.5 ng/ml) for more than 24 days. Accuracy of the clinical dignosis for the corpus luteum, by transrectal palpation before the first postpartum estrus was 44.6% (supported by a high detected level of progesterone). After the first postpartum estrus accuracy of corpus luteum diagnosis increased to 84.7%. The interval between estrus and the appearence of a high level of progesterone (≥ 0.5 ng/ml) was 7.4 ± 3.6 days.

FIG. 1 shows different progesterone profiles observed during the postpartum period in crossbred primiparous cows.

DISCUSSION

The animals calved and showed signs of estrus with 85% and 90% of their adult weight, respectively. In primiparous crossbred cows a significant correlation (R=0.44 P<0.05) be-

tween weight at estrus and change of weight calving to estrus interval was observed. This was not similar to those reported by Peters and Riley [15], who reported that change in postpartum weight is apparently not related to the postpartum acyclic period.

Progesterone secretion prior to the first postpartum estrus was observed in 50.8% of the cows. Similar finding has been reported by several researchers [2, 9, 12, 25]. The duration of P4 secretion was less than ten days in 87.1% of the animals and was also of a low level. These results are in agreement with those of the other studies which have reported P4 cycles of variable levels but of short length [2, 10, 11, 15, 23]. This first P4 secretion has been postulated to be the product of the luteinized follicles [23, 25]; corpora lutea of short lifespan coming from an ovulation without signs of estrus [6, 10, 22], poor formation of the corpus luteum due to a lack of the LH peak [19]; incomplete follicular development with poor steroidogenic function and low number of ovarian LH receptors [9]; or poor blood suply of the first corpus luteum [19]. The results indicated that only 54.8% of the cows showing the first P4 secretion had a clinical diagnosis of a corpus luteum. Progesterone secreted by luteinized follicles from a poorly developed corpus luteum or an unknown cellular source may account for this secretion during the postpartum period [1, 5].

The existence of dissociation between ovulation and the absence of behavioral estrus (silent heat) was confirmed in this study and is in agreement with several published reports [9, 19, 23]. A high silent ovarian activity rate (occurrence) has been reported in highly-producing cows [8]. This was confirmed in this study as 70% of the crossbred Holstein cows with high production showed the same hormonal pattern and behavior. Estrus signs without ovulation have also been reported [19]. In the sample studied, anovulatory estrus (10.9%) was more frequently observed when occurring the first 45 days postpartum. This is agreement with [19]; however, these authors reported an incidence of 24% anovulatory estrus during the first postpartum days. Estrus during the luteal phase, prolonged progesterone levels for more than 25 days, and supression of the ovarian activity after the first postpartum progesterone secretion were detected in this study as has been reported by others researchers [4, 8, 9, 19]. Accuracy between high P4 level and clinical diagnosis of a corpus luteum was more exact after the first postpartum estrus (84.78%) than before (44.64%). An accuracy of the 77% has also been reported [3, 14]. These findings suggest that the appereance of the first postpartum estrus is generally followed by formation of a functional corpus luteum. They also suggest that high P4 levels before the first postpartum estrus could originate from a different source than a functional corpus luteum.

This fact might explain the high diagnosis error during the early postpartum period. This reproductive biological phenomenon demands more research effort in order to define its physiological importance and its relationship to the resumption
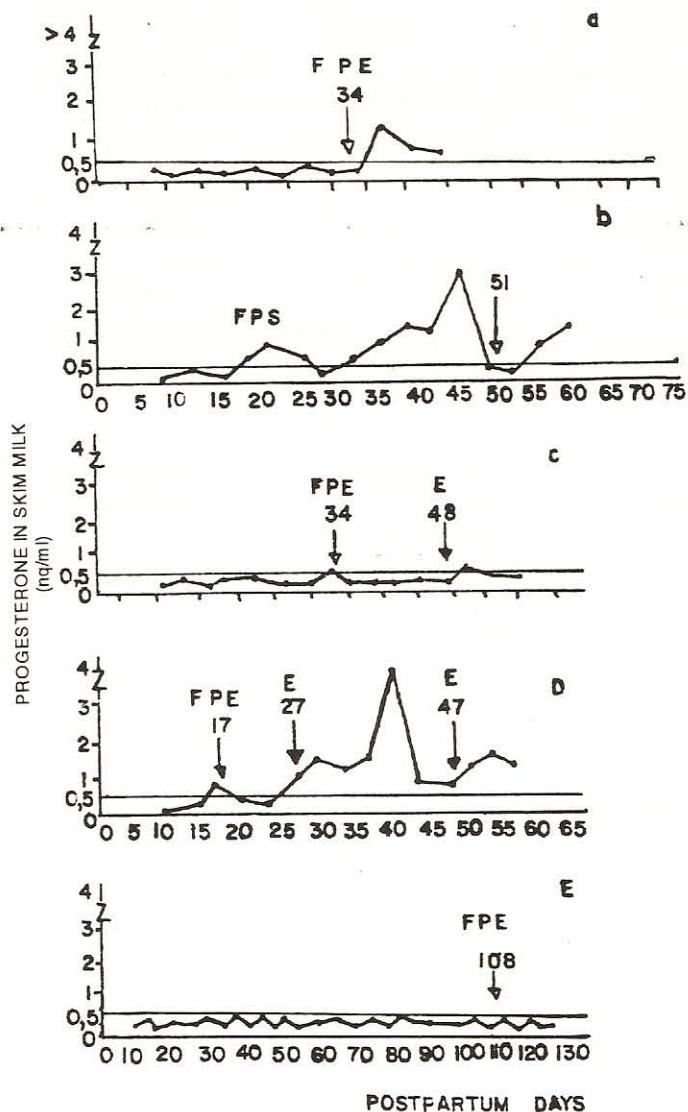

FIGURE 1. POSTPARTUM PROGESTERONE PROFILES. a) OVULATORY ESTRUS; b) FIRST PROGESTERONE SECRETION (FPS); c) ANOVULATORY ESTRUS; d) ESTRUS IN LUTEAL PHASE; e) ANESTRUS COW AND ANOVULATORY ESTRUS. FPE: FIRST POSTPARTUM ESTRUS. E: ESTRUS.

of the postpartum ovarian activity (including ovulation and exhibition of the estrus behavior) [2, 15, 17].

The results also showed that within 60 days postpartum 70% of the cows were cycling. Although it is lower than those reported for purebred dairy cattle in a temperate climate [2, 9]. However, it has to be considered satisfactory for crossbred animals under tropical conditions. The high incidence of anestrus, acyclic cows (27%) suggest the need to establish more intensive reproductive managment programs to control this group of problem cows. The interval between estrus and appereance of a progesterone level ≥ 0.5 ng/ml was similar to the reported [19].

In primiparous crossbred cows the presence of an estrus ovulation dissociation was most frequent before the first postpartum estrus. The anovulatory estrus had a low incidence. These animals also showed a first progesterone secretion before the first postpartum estrus whose origin and physiological significance should be better defined. The first postpartum estrus was a better indicator of the postpartum ovarian activity than changes in the progesterone profiles: Therefore, a good estrus detection program for the first postpartum estrus is of vital importance under tropical conditions and should be used to implement an early postpartum breeding program in order to improve reproductive efficiency. Under these tropical conditions, the mechanical milking and the feeding supplementation in nonsuckling crossbred primiparous cows, an early postpartum ovarian activity was observed which makes possible the shortenig of the interpartum interval.

ACKNOWLEDGEMENTS

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