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Restricted Suckling and Hormonal Treatment on the Postpartum Ovarian Activity Resumption of Beef Cows in the Tropics of Mexico

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Abstract: The aim of this study was determined the effect of suckling of the calf restricted to once a day per 30 min plus a combined hormonal treatment on the postpartum anestrous period and fertility in beef cows after 120 days postpartum. About 67 *Bos taurus* x *Bos indicus* crossbred multiparous pregnant cows were used and randomly distributed to five treatments: traditional suckling (TS; n = 15), calf stayed all day with the dam; restricted suckling (RS; n = 14), calf sucked the cow once a day per 30 min beginning day 7 postpartum; TS plus a combined hormonal treatment (TSH; n = 15), applied at day 50 postpartum, consisting in GnRH+Norgestomet for 7 days+$PGF_{2\alpha}$ (day 0)+GnRH (day 2); RS plus hormonal treatment (RSH; n = 16) as described in group 3 and without suckling (WS; n = 7) which included cows with death calves before day 7 postpartum. There was an increase ($p \geq 0.001$) in the ovarian activity of cows in the RS and RSH treatment compared to TS and TSH group cows (78.6 and 87.5 vs. 26.7 and 46.7%, respectively). Calving-first ovulation interval for WS cows was shorter ($p \leq 0.001$) than intervals for RSH, RS, TSH and TS cows (22.3 vs. 49.3, 68.8, 63.5 and 86.3 days, respectively). Likewise, the calving-first ovulation interval was shorter for RSH ($p \leq 0.01$) vs. TSH and TS. Cows in RS and RSH treatments were more fertile ($p \leq 0.001$) than those from TS and TSH groups (42.3 and 56.3 vs. 20.0 and 6.6%, respectively). The WS cows (71.4%) had higher fertility ($p \leq 0.001$) than cows from all other combined treatments (31.6%). No differences were found between treatments ($p > 0.05$) regarding the incidence of corpora lutea of short half-life. In conclusion, suckling restricted to 30 min reduced the resumption period of ovarian activity in beef cows in the tropics of Mexico. Double dose of GnRH plus Norgestomet and $PGF_{2\alpha}$ induced fertile ovulations after 50 days postpartum in restricted suckling cows.

Key words: Restricted suckling, postpartum, GnRH, progestogen, $PGF_{2\alpha}$, resumption

INTRODUCTION

Anestrous postpartum is the main cause of infertility in cows (Short *et al*., 1990) and their length can be influenced by the interaction between social (cow-calf link) and lactation factors (Berardinelli *et al*., 2005) (Quintans *et al*., 2009) because when the sensibility of the negative feedback of estradiol 17β from the ovary increases, failures occur in ovulation and also increases the period of resumption of ovarian activity (Yavas and Walton, 2000). Once the postpartum anestrous is linked to suckling, one way to promote the onset of the ovarian activity is to wean the calf, either fully (early weaning), temporary (48-96 h) or partially (restricted suckling once or twice per day) in either case the release of GnRH (Gazal *et al*., 1999) is increased, increasing the frequency of LH pulses (Hoffman *et al*., 1996; Stagg *et al*., 1998) and the concentration of LH and FSH receptors (Walters *et al*., 1982). These responses are followed by ovulation within the next days (Bell *et al*., 1998).

Studies have been carried out on anestrous cows treated with GnRH, a progestagen and the removal of the calf reducing the incidence of corpora lutea of short half-life (Troxel *et al*., 1993).

When the application of GnRH precede that of the progestagen by 7 days, plus $PGF_{2\alpha}$, pregnancy rates have been increased in suckled cows (Forbes *et al*.,

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1997; Stevenson *et al*., 1997) indicating that without the withdrawal of the calf, GnRH plus the progestagen can restore the normal luteal activity reducing the incidence of short half-life corpora lutea. Previous studies have shown that when ovulation is synchronized in suckled cows using GnRH, Norgestomet and $PGF_{2\alpha}$ with artificial insemination, 16 h after the second application of GnRH, pregnancy rates similar to the control cows inseminated 12 h after estrous detection were found (Thompson *et al*., 1997). Several methods have been studied to synchronize estrous in cattle such as the use of a progestagen, $PGF_{2\alpha}$ and the combination of both (Odde, 1990; Twagiramungu *et al*., 1992).

Although, the estrous has been synchronized, the use of those methods has not improved fertility to the desired levels. Improving the presentation of estrous depends on the control of the development of the follicle and subsequently of the corpus luteum (Fogwell *et al*., 1986). This concept was confirmed by previous studies (Sirois and Fortune, 1990) which indicate that in response to the luteolysis induced by $PGF_{2\alpha}$, the preovulatory peak synchrony of LH depends on the homogeneity of the population of preovulatory follicles at treatment time. In addition, small doses of progestagens in the absence of a corpus luteum increases the frequency of pulses of LH, the occurrence of persistent dominant follicles and concentrations of estradiol-17β however, fertility has been reduced (Sanchez *et al*., 1995; Smith and Stevenson, 1995).

In tropical areas the lack of the timely-detection of estrous is an obstacle to efficiently implement the use artificial insemination during controlled mating, therefore, the objective of this study was to determine the effect of a hormonal treatment on the resumption of postpartum ovarian activity, half-life of corpus luteum and fertility rate of crossbred zebu cows under to two types of suckling.

MATERIALS AND METHODS

Study area: The research was carried out in the Campus Tabasco of the Colegio de Postgraduados located in Cardenas, Tabasco, Mexico at 18°00' North latitude and 93°30' West longitude and 9 m above sea level. The climate of the region is Am type, warm and humid according to the classification of Koppen with average annual rainfall of 2,231 mm and average annual temperatures of 26.1°C.

Animals and treatment: A total of 67 *Bos taurus* x *Bos indicus* crossbred multiparous pregnant cows with an average age of 6.38±2.4 years and a live weight of 446.8±58.7 kg, respectively were randomly distributed as they calf to the following treatments: traditional suckling (TS; n = 15): Cows with the calf all the time; restricted suckling (RS; n = 14), calf sucked the cow once a day per 30 min beginning day 7 postpartum; TS plus a combined hormonal treatment (TSH; n = 15). About 50 days after calving they were applied (μm) 100 g of an agonist Gonadotrophic Releasing Hormone (GnRH), an implant with 6 mg of norgestomet placed s.c. on the posterior aspect of the ear and removed for 7 days later (day 7). After removal of the implant, 25 mg of prostaglandin $F_{2\alpha}$ ($PGF_2\beta$; day 0) was applied (μm); 48 h later a second dose of 100 μg of GnRH (day 2) was applied (μm), the cows being inseminated 24 h later (day 4) RS plus hormonal treatment (RSH; n = 16) as described in group 3 without suckling (WS; n = 7) which included cows with death calves before day 7 postpartum. The experimental design was a completely randomized design with a 2×2 factorial arrangement of treatments plus a control.

Animals grazed together in Star African (*Cynodon plectostachius*) paddocks with native grass (*Paspalum* sp.) and had water *ad libitum*. Estrous was detected by visual observation twice a day (morning and afternoon) for an hour, registering the date and hour of estrous detection and artificially inseminating the cows 12 h after estrous detection or at the time predetermined in the hormonal treatment of synchronized ovulation.

Progesterone determination and resumption of the postpartum ovarian activity: From day 7 postpartum, a blood sample from each cow was collected from the jugular vein, twice a week until day 120 postpartum. Samples were centrifuged at 1,800×g and -4°C for obtaining the sera which were stored at -20°C until progesterone determination by Radioimmunoassay (Coat-A-Count, Diagnostics Products Corporation), with a sensitivity of 0.1 ng mL^{-1} and a coefficient of variation of 3.93%.

Ovulation was assumed (Stevenson and Call, 1983) when 2 days before, an increment of progesterone >1 ng mL^{-1} but <2 ng mL^{-1} was detected 4 days before, increments of progesterone (≥2 ng mL^{-1}) with respect to the mean base serum concentration was observed.

The length of the luteal phase was determined by the number of consecutive days that serum concentrations of progesterone were ≥1 ng mL^{-1}, classifying the luteal phases into short when the half-life of the corpus luteum was <14 days and normal when it was ≥14 days.

Statistical analyses: Data of the categorical variables, resumption of ovarian activity, half-life of the corpus luteum and fertility were analyzed using binary logistic regression procedures.

Also χ^2-tests were carried out to compare the control treatment (WS) against the other four treatments. For data on days at first ovulation postpartum, survival analysis was used (JMP, 2002) being the cow the experimental unit.

RESULTS AND DISCUSSION

Resumption of postpartum ovarian activity: Neither hormonal treatment nor interaction effects were observed ($p>0.05$) on resumption of the ovarian activity after 120 days postpartum. However, there was an effect of type of suckling ($p<0.001$). A higher rate of ovarian activity was observed in the restricted group of cows as compared to the group of cows which have their calves 24 h (Table 1).

First postpartum ovulation: The survival curves (Fig. 1) show a gradual decrease in the number of days to first postpartum ovulation due to the effect of the suckling type. Anestrous period was longer for the TS and TSH group cows and shortest for the WA group. No differences were found ($p>0.05$) between RS and RSH treatments (49.3±4.6 and 68.8±11.1 days, respectively) as well as among TSH, RSH and TS treatments (63.5±3.79, 68.8±11.1 and 86.3±7.97 days, respectively). The average calving-first ovulation interval for the WA group (22.3±4.34 days) was lower ($p<0.001$) compared with the other groups as was RSH ($p<0.01$) compared with TSH and TS (Table 2).

Fertility rate: No effect of hormonal treatment or interaction of hormonal treatment and type of suckling ($p>0.05$) on pregnancy rate was found (Table 2) however, restricted suckling treatments (RS and RSH) had higher fertility ($p<0.001$) compared with traditional suckling (TS and TSH). In addition, the WS group (72.4%) had higher fertility ($p<0.001$) than all other experimental groups (Table 3).

Half-life of corpus luteum: No effect of type of suckling, hormone treatment or their interaction was found ($p>0.05$) on half-life of the corpus luteum (Table 1 and 3).

Results suggest that cows not being suck or under restricted suckling had first postpartum ovulation intervals shorter than cows with the calf all the time. Also the hormonal treatments had a synergy effect decreasing the anestrous postpartum period. These data agree with

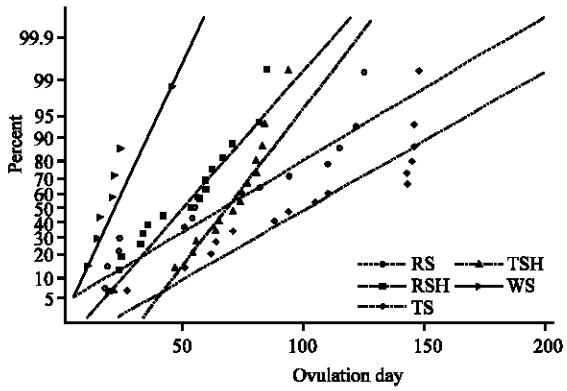

Fig. 1: First postpartum ovulation survival curves of zebu crossbred cows under Traditional Suckling (TS), suckling restricted to 30 min day^{-1} (RS), TS plus a hormonal treatment (GnRH-Norgestomet-$PGF_{2\alpha}$-GnRH) 50 days postpartum (TSH), RS plus hormonal treatment (RSH) and cows without being suck (WS)

Table 1: Resumption of the ovarian activity, half-life of the corpus luteum and fertility after 120 day postpartum of zebu crossbred cows under different suckling types and hormonal treatments

				Half-life of the CL		Fertility	
Groups	N	n	Ovulating cows (%)	<14 day n (%)	≤14 day n (%)	n	%
TS	15	4	26.7^{b}	2 (50.0)	2 (50.0)	3	20.0^{d}
RS	14	11	78.6^{a}	6 (54.5)	5 (45.4)	6	42.9^{c}
TSH	15	7	46.7^{b}	6 (85.7)	1 (14.2)	1	6.7^{d}
RSH	16	14	87.5^{a}	7 (50.0)	7 (50.0)	9	56.2^{c}

*Values with different literal in the same column differ significantly ($p<0.01$)

Table 2: Time to first postpartum ovulation of *B. taurus* x *B. indicus* crossbred cows under Traditional Suckling (TS), suckling restricted to 30 min day^{-1} (RS), TS plus a hormonal treatment (GnRH-Norgestomet-$PGF2_{2\alpha}$-GnRH) 50 days postpartum (TSH), RS plus hormonal treatment (RSH) and cows without being suck (WS)

Groups	N	No. cows censored	Fist postpartum ovulation (days)*
TS	15	11	86.26±7.970^{c}
RS	14	3	68.83±11.08^{bc}
TSH	15	8	63.53±3.790^{c}
RSH	16	2	49.25±4.600^{b}
WS	7	0	22.28±4.340^{a}

*Values with different literal in the same column differ significantly ($p<0.01$)

Table 3: Resumption of ovarian activity, half-life of the corpus luteum and fertility after 120 days postpartum of zebu crossbred cows with Traditional (TS) and Without Suckling (WS)

	Ovulating cows*			Half-life of the CL		Fertility*	
				<14 day	≤14 day		
Groups	N	n	%	n (%)	n (%)	n	%
TS	60	36	60[b]	21 (58.3)	15 (41.6)	19	31.60[d]
WS	7	7	100[a]	4 (57.1)	3 (42.8)	5	71.42[c]

*Values with different literal in the same column differ significantly ($p<0.01$)

previous results (Amaya *et al*., 2007) where the application of GnRH and $PGF_{2\alpha}$ in lactating cows without a calf reduced the first postpartum ovulation interval. Suckling once a day per few minutes reduces the inhibiting effect of the presence of the calf on resumption of ovarian activity (Stagg *et al*., 1998). On the other hand, the hormonal treatment of GnRH-norgestomet-$PGF_{2\alpha}$-GnRH 50 days postpartum was late for the RS treatment because 7 of the cows belonging to that group had already ovulated previously. In the case of the TS group plus hormonal treatment it is appreciated that the hormone treatment was not effective to block the inhibitory effect of the presence the calf and in consequence the first ovulation occur 25 days after treatment. However, notwithstanding that the onsets of the restriction periods are different, the results obtained in this study agree to those reported by other researchers (Hoffman *et al*., 1996; Stagg *et al*., 1998; Browning *et al*., 1994) who indicated that the RS once a day per 30-90 min shortens the anestrous postpartum.

The pregnancy rate (56.3%) of the RSH group cows was similar to that obtained in previous studies (Twagiramungu *et al*., 1995) where a similar protocol was used but without the use of Norgestomet (GnRH-$PGF_{2\alpha}$-GnRH) and artificial insemination 54 h after for the second application of GnRH.

Also, in other study (Schmitt *et al*., 1996), estrous synchronization of 94 cycling heifers using the $PGF_{2\alpha}$-CIDR-B-GnRH-$PGF_{2\alpha}$ scheme obtained 60.6% of fertility. As is noted the lowest percentages of fertility were for cows under TS treatments. This was probably due to the fact that these groups had the lowest rates of response at the onset of the ovarian activity in the first 120 days postpartum and on the other hand, the group that received the hormonal treatment 50 days postpartum (TSH) which was lowest of the all had high incidence of corpora lutea of short half-life (85.7%) after the first postpartum ovulation that was induced by the combined hormonal treatment.

On the other hand, the incidence of corpora lutea of short half-life observed in this study, agree with those obtained by other researchers (Perry *et al*., 1991; Crowe *et al*., 1993) who report a high incidence after the first postpartum ovulation.

CONCLUSION

Suckling restricted once a day per 30 min after day 7 postpartum reduced the resumption of ovarian activity of beef cows. Treatment with GnRH-Norgestomet-$PGF_{2\alpha}$-GnRH applied after 50 days postpartum did not block the inhibitory effect of TS on the pulse generator of GnRH and induce the first postpartum ovulation. Restricted suckling plus the hormonal treatment applied 50 days postpartum was able to synchronize and induce fertile ovulations.

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