Influence of suckling time and calving trimester on postpartum ovary activity retake in Bufalipso crossbreds

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Thirty-four crossbred Bufalipso (*Bubalus bubalis*) cows were studied. They were multiparous, and had weights and ages between 450-600 kg and 8.8-12 years, respectively. Two experimental groups were formed, according to the suckling times after the milking. The first group remained with the calves one hour after the end of the milking (A, n=17), while the second was kept six hours later (B, n=17). The second study consisted in evaluating the effect of the calving trimester on the reproductive performance. The animals that calved in June, July, and August formed the first group (T1, n=18), while the second grouped the calvings occurred in September, October, and November (T2 n= 16). Every 72 h, each ovary was diagnosed through transrectal ultra-sonography. An analysis of variance was performed, using a linear model and with adjustment of covariance for the calving number. The first dominant follicle (days), as well as the first and second corpora lutea (days) for the A and B treatments were: 15.81 and 21.11 ($P < 0.05$), 26.43 and 32.13 ($P < 0.01$) and 42.93 and 45.50 ($P > 0.05$), respectively. For T1 and T2, they were of 20.14 and 16.78 ($P < 0.05$), 45.00 and 32.56 ($P < 0.05$), and 58.21 and 44.23 ($P < 0.05$), respectively. It was concluded that the suckling effect affected the appearance of the first dominant follicle and the first corpus luteum, but did not take part in the appearance of the second, or in the reproductive interval under study. The months of September, October, and November showed, significantly, an earlier retake in all the indicators, compared with June, July, and August, respectively.

Key word: *reproductive intervals, ovary activity, river buffalo cows.*

The early retake of the postpartum ovary activity is an important element to obtain an economically wanted reproductive efficiency. However, there are factors affecting this reproductive performance, involving the feasibility of the productive cycles. Suckling has been a practice commonly used in buffalo herds as stimulus to the milking. However, this behavioral and physiological phenomenon affects the early retake of the postpartum ovary activity, affecting the reproductive behavior (El Wishy 2007).

Many researches relate the influence of the calving season to several aspects of the reproductive behavior. It has been proved how changes are produced in the responses at the endocrine level and, thus, differences in the retake of the postpartum ovary activity. Although the domestic buffalo is considered as having seasonal behavior with multiple estruses of short days, in tropical regions, the seasonal reproductive behavior is due, primarily, to nutritional factors and heat stress (Vale 2007).

The study of the postpartum ovary activity, incorporating the partum season effect and different suckling systems will allow deeper knowledge on the physiological mechanisms emerged during the puerperium. Besides, it contributes to clarify questions arising from artificial insemination, where there is, in general, low technical efficiency, due to difficulties in estrus detection, along with the determination of the ideal time for the application of the hormonal protocols in the synchronization and artificial insemination programs at fixed time.

The objective of this study was determining the retake of the postpartum ovary activity through two post-milking suckling times and different calving trimesters, using as diagnosis tool the transrectal ultrasonography in real time and B mode.

Materials and Methods

Experimental units. The work was performed from July to November in 2009 at the experimental buffalo station of the Institute of Animal Science. A total of 34 river buffalo cows were used. They were multiparous and had weight and age of 450-600 kg, and 8.8 to 12 years, respectively. The average production of the animals ranged from three to four milk liters. They had body condition score of 3.5 to 4.5 points, according to the estimation of Houghton *et al*. (1990).

Management and feeding. The animals were used in semi-intensive form. The fundamental feeding was based on natural pastures, such as Indian bluegrass (*Botriochloa pertusa*) and Bahia grass (*Paspalum notatum*); besides cultivated pastures. There was predominance of CT-115 (*Pennisetum purpureum*) and star grass (*Cynodon nlemfuensis*). The milking was performed manually and with support of the buffalo calf, leaving a quarter without milking; besides the residual milk. The buffalo cows, located in shade pens, were fed commercial concentrate with 16 % of CP, at a rate of 2 kg/animal/d. Water and mineral salts were given *ad libitum*.

Procedure and experimental design. In order to evaluate the suckling effect, two experimental groups were formed, according to lactation time and later milking. The first group (A, n=17) remained with the calves one hour after the end of the milking, whereas the second remained for six (B, n=17). After each test time, both categories were separated and located in grazing paddocks up to the next milking.

The second effect under study for the reproductive behavior was the calving trimester. The animals with the calves during June, July, and August formed the first group (T1 n=18), whereas the second was composed of those that calved in September, October and November (T2, n= 16).

With a 72-hour interval, and since the 10 and up to 60 d postpartum, in both effects, the ovary activity was evaluated through transrectal ultrasonography, in real time and B mode (Aquila Pro, Pie Medical, Netherland), using a linear transducer of 7.5 MHz, according to the methodology of Dahiya *et al*. (2003). Throughout the study, the ecography was conducted by the same operator. The time of appearance (d) and the diameter (mm) of dominant (DF) and pre-ovulation (POF) follicles were determined, as well as the presence of corpora lutea (CL).

The follicles were classified according to their diameter, into small (<4.5 mm), middle (>4.5 mm and <8.5 mm) and large (>8.5 mm), according to Lohan *et al*. (2004). All the ovary structures visible to ultrasonography were identified and recorded based on data for later statistical processing.

An analysis of variance was conducted, using a linear model and adjustment of covariance for the calving number. The effects of treatment, calving trimester and interaction were included. The means were transformed according to the square root of the original data. The test of Duncan (1955)was applied for P < 0.05. The statistical software was INFOSTAT, version 1 (2008).

Results and Discussion

Table 1 shows the performance of the early ovary activity. In both suckling systems, there were significant differences between the variables. However, there were not present in the appearance of the second corpus luteum. These results confirmed the report of DeRensis *et al*. (1993), who noted that, in time, there was gradual increment of the pulses and concentrations of the gonadotropic hormones.

In respect to the appearance of the first dominant follicle and its diameter (tables 1 and 2), the results for the A1 group agreed with those of Kozicki and Crespi (1997), who located it at the day 14.6 ±6.8, using restricted suckling system. The interaction between the effects under study and the diameter of the first dominant follicle proved significance favoring the restricted suckling system during the calving trimester (June- July- August), which was in agreement with the studies of Lohan and Matik (2004) in restricted systems where more than 50 % of the animals had their follicles as dominant, with

Table 1. Postpartum ovary activity through different suckling systems

Variables	Treatments				SE± Sig.	Sig. Cov.
	Restricted suckling	N	Non restricted suckling	n		
1 DF (days)	3.95 (15.81)	17	4.56 (21.11)	17	0.13*	NS
1POF (days)	4.78 (23.48) ±0.68	13	5.88 (30.07) ±0.62	14	**	**
Diameter (mm)	1.27 ±0.04	13	1.15 ±0.03	14	*	NS
1CL (days)	5.07 (26.43) ±0.73	13	6.03 (40.13) ±0.59	13	**	**
2DF (days)	5.4 (29.63) ±0.16	15	5.93 (37.44) ±0.16	16	*	NS
Diameter (mm)	1.11 ±0.03	15	0.98 ±0.02	16	*	NS
2POF (days)	5.88 (35) ±0.17	9	6.43 (41) ±0.15	11	*	NS
Diameter (mm)	1.37 ±0.04	9	1.22 ±0.03	11	*	NS
2CL (days)	6.36 (40.93) ±0.19	9	6.66 (45.50) ±0.22	7	*	NS

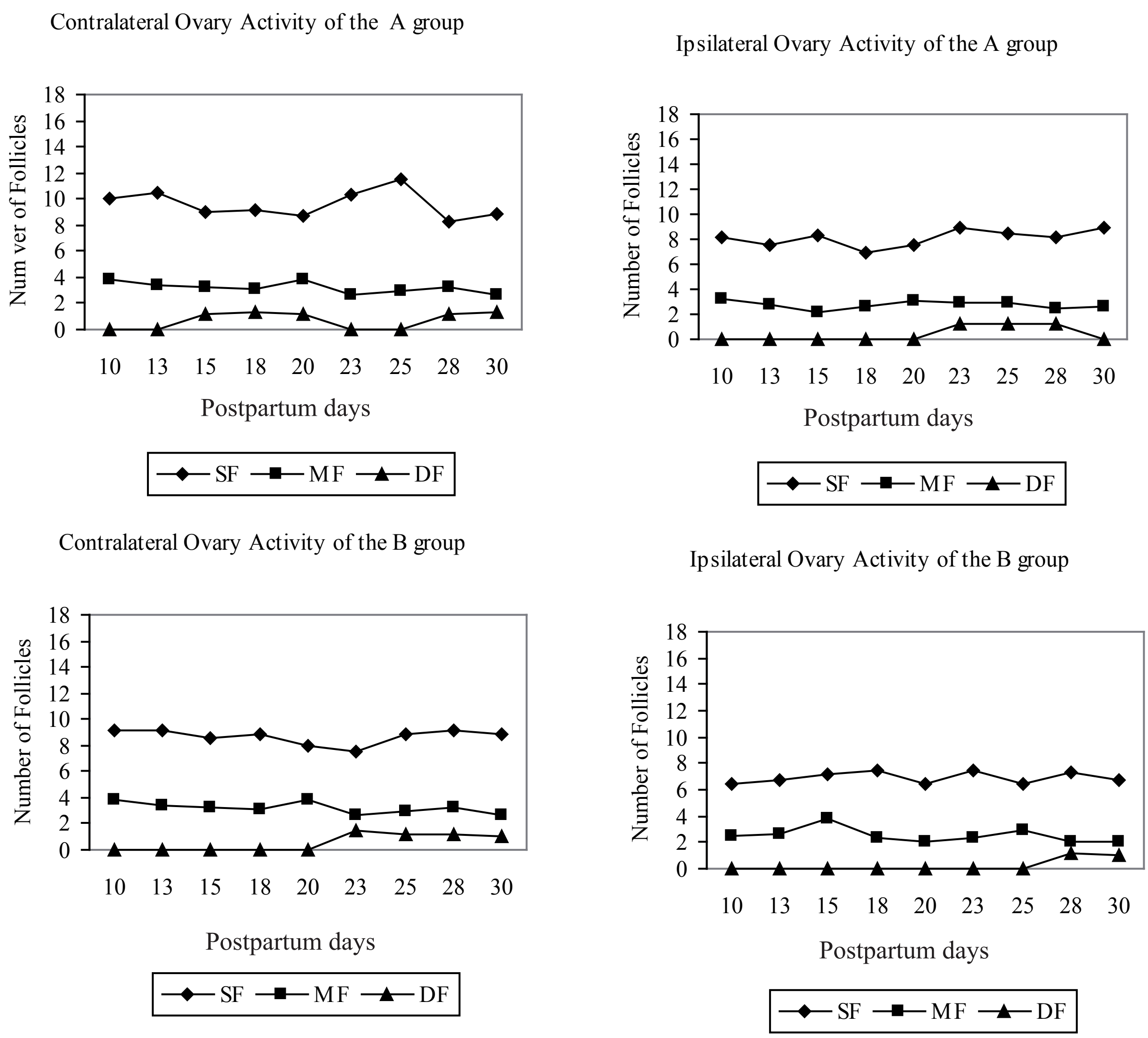
( ) Original means. * P < 0.05 * *P < 0.01

diameter ≥ 8.5 mm, since 21 d postpartum. These values were in agreement with the mean of the animals from the A2 group. However, they were superior to those from the A1 group. Likewise, Tiwari *et al*. (1993) proved that the apperance of follicles with diameter ≥ 10.0 mm at 22 and 30 d, in artificial and natural rearing conditions, respectively. In the groups under test, the differences could be related to the effect of prolactin on the blood during the suckling period. It takes part negatively in the retake and early development of the postpartum ovary activity. Nevertheless, as this stimulus decreases, there is gradual rise of the hypothalamus-hypophysis-gonad activity. The fit of covariance for the calving number affected significantly the appearance of the first preovulatory follicle and the first corpus luteum in both groups under study, whereas in the rest of the variables it had not effect. This also proves as in other species that younger animals, compared with adults, delayed their early postpartum ovary onset due to the need for using part of the nutrients in the finishing of the body growth

Table 2. Postpartum ovary activity through different calving season

Treatments	Trimester	
	June-july-august	Sep-oct-nov
Restricted suckling	1.07^b (9)	0.92^a (8)
	±0.03	±0.03
Non restricted suckling	0.92^a (9)	0.95^a (8)
	±0.03	±0.03

( ) Original means
* P < 0.05 * *P < 0.01



SF- Small follicles, MF- Middle follicles, DF- Dominant follicles

Figure 1. Retake of the postpartum follicular activity in the ovary contralateral and ipsilateral to the gravid horn of the previous pregnancy, through different suckling periods.

and development.

In both groups, the highest follicle activity (figure 1) was in the ovary contralateral to the gravid uterine horn from the previous pregnancy. Likewise, the studies of Usmani (1992) confirmed that during the first thirty days there was higher follicle intensity. In respect to the diameter of the pre-ovulation follicles, there were differences as compared with reports by other researchers (Presicce *et al*. 2005 and Rubio *et al*. 2006), who found a diameter of 14 mm in multiparous buffalo cows, without standing calves. In general, these values could be due to the suppressing effect of the suckling, in respect to the frequency and magnitude of the pulses of the LH, main hormone affecting the final growth and ovulation of the dominant follicle.

In both suckling systems, the number of follicles recruited per each cycle of follicle growth was low, similar to Gimenes *et al*. (2009), cited by Baruselli (2010). This could account for the low super-ovulation response of this species (Chunyan *et al*. 2010). As to the first postpartum ovulation, the studies in different regions demonstrated wide variability, in aspects such as the poor nutrition, the climate, the inadequate management and the continuous suckling, among others (Campo *et al*. 2000).

In this study, 88.2 and 76.5 % of the animals, for the A1 y A2 groups, showed their first postpartum ovulation ($P < 0.05$) at 26.43 ± 5.07 (17-45 d) and 32.13 ± 6.3 (23-55d) d, respectively.

These values were within the ranges obtained by Perera *et al*. (1987) in herds managed with restricted suckling, contrary to the herds with free suckling, which had values ≥ 150 d. Likewise, reports of El-Wishy (2007) disagreed notably with ours. This author noted that in free suckling systems, between 34 and 49 % of the animals, started their ovary activity during the first 90 d, whereas 31 and 42 % were above 150 d.

In respect to the technologies of partial restriction or continuous suckling, the intensive production systems, with artificial rearing of the buffalo calf, enhance notably the reproductive behavior, so was proven by the works of Presicce *et al*. (2005), where 62.5 % of the buffalo cows showed the first corpus luteum at 15.5±1.3 d, with a range of 8-20 d. These records were inferior to ours, which proved the suckling effect and the endogenous opioids when releasing hypophysis gonadotropins and the early restore of the ovary activity.

Table 3 provides the average values of the follicle growth, according to the calving season. During September, October and November the first pre ovulatory follicle appears much earlier ($P < 0.05$), as well as the fist corpus luteum which could be the cause for the folllicle diameter were smaller, compared with June, July and August ($P < 0.05$), due to gradual postpartum recovery of the stimulant and luteinizing follicle hormones. For both variables and similar to the suckling systems under study, the fit of covariance affected significantly the calving number, which proved that regardless the calving season, this condition affected the early onset of the follicle developement.

Similar results were found by Campo (1991), who noted since the third and fourth week week, increment in the number of follicles ≥ 0.5 mm in the period from November to February, compared with that from May to September. This author reported some aspects, such as the temperature-humidity index (THI) and the body condition at calving, which could affect notably the postpartum ovary growth. Similarly, Capitan and Takkar (1988) found early start in winter (24 d) compared with summer (34 d). However, non-significant differences were found by Bahga *et al.* (1988), which could be due to the best feeding of these herds, in a way that the climatic effects on the reproductive behavior could be compensated.

In respect to the reproductive intervals under study, it was proven that the suckling effect, according to the times, did not affect the postpartum reproductive behavior, because there were not differences when analyzing the calving-pregnancy (CPI) and the calving-calving intervals (CCI). They were, for the A1 y A2 groups, of

Table 3. Postpartum ovary activity in different calving seasons.

Variables	Trimesters				SE ±	Sig.
	J-J-A	n	S-O-N	n	Sig.	Cov.
1 FD (days)	4.45 (20.14) ± 0.13	18	4.06 (16.78) ± 0.13	16	*	NS
1 FPO (days)	5.78 (33.59) ± 0.62	14	4.88 (24.89) ± 0.67	13	**	**
Diameter (mm)	1.26 ± 0.04	14	1.16 ± 0.03	13	*	NS
1 CL (days)	6.23 (39.00) ± 0.58	14	5.15 (27.56) ± 0.73	12	**	**
2 FD (days)	5.83 (34.38) ± 0.16	15	5.5 (30.69) ± 0.16	16	*	NS
Diameter (mm)	1.05 ± 0.04	15	0.97 ± 0.02	16	*	NS
2 FPO (days)	6.45 (41.85) ± 0.15	11	5.85 (34.65) ± 0.17	9	*	NS
Diameter (mm)	1.35 ± 0.04	11	1.24 ± 0.04	9	*	NS
2 CL (days)	6.79 (46.21) ± 0.17	8	6.24 (39.23) ± 0.21	8	0.20*	NS

() Original means. *$P < 0.05$ **$P < 0.01$

Figure 2. Reproductive intervals through different calving seasons

40.63±3.65, 42.11±3.86, 357.75±12.63 362.16±13.23 d, respectively. In general, these values were in the range reported by Martínez (2006). Hovwever, they did not agree with those of Ramos *et al*. (2001), who found superior values. These differences could be explained by the existing conditions, because these experiments were performed in poorly drained prairies, where pasture availability and quality in respect to our medium could negatively affect the nutrition.

As to the calving trimesters and the reproductive intervals (figure 2), September, October and November showed lower values compared with June, July and August, being 42.27±4.69 and 72.82±5.96 for CPI, and 355±12.33 and 381.43±13.63 for CCI ($P < 0.05$). These results were in accordance with those of Campo (1991); however, they were not in correspondence with those of Méndez *et al*. (2009) in a study in the eastern region of Cuba, where the best reproductive behavior was in the animals that calved in the june-july-august trimester. This could be, mainly, due to the environmental conditions, specifically those related to the rainfall levels and the forage availability during the rainy season, which in our region occurs with higher intensity and length. This permits that from September to November other factors may be associated, such as the fall in temperature, relative humidity and light hours. Besides, the last pregnancy trimester flows with superior feeding, and the calving occurs with better body condition of the cows. These conditions were in correspondence with Arias *et al*. (2006), who stated that buffalo cows with good management and feeding have an early retake of the ovary activity.

In respect to the detection of the second corpus luteum and its relation to the suckling effect and the year trimester (table 1 and 2), they agreed with the average values of the calving-pregnancy period, which confirmed that most of the animals were born during this stage. This proved the excellent behavior and adaptation degree of this species. In general, the CCI, for each treatment, was between 13 and 14 months, period in which it is considered efficient, in climatic conditions similar to those of this study (Albuquerque 2010).

It was concluded that the suckling effect on the systems under study did not affect the early retake of the postpartum follicular growth, as well as on the diameter of the dominant and pre-ovulation follicles. Nevertheless, it had no consequences on the appearance of the second corpus luteum or on the reproductive intervals under study. In respect to the calving trimester, there were differences favoring the September-october-november trimester, as to the retake of the ovary activity, as well as to the presence of the first and the second corpora lutea, besides the calving-calving and calving-pregnancy reproductive intervals, compared with the june-july-august trimester. Further research is recommended to attain deeper knowledge on aspects related to the management of this species and the climatic effects that may influence the productive behavior.

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