

# Productive and Reproductive Parameters in Dairy Cows and the Relationship with Ovarian Follicular Dynamics in the Puerperium

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**ABSTRACT---** *The study aimed to evaluate follicular dynamics in postpartum dairy cows and to correlate several productive and reproductive characteristics with the pregnancy rate. Ultrasonographic examinations were performed on 34 Holstein cows every 5 days starting on day 5(d5) to d40 pp. Data regarding dominant follicles(DF), ovulatory follicles (OF), ovulation frequency, corpus luteum diameter, luteal phase length, and other parameters were obtained. DF were observed in 17.6% of animals on d5 pp, and the incidence increased ( $P<0.05$ ) at d10 and thereafter. The average diameters of the OF and corpus luteum were 21.0 and 23.3 mm, respectively. After 40 days, 67.7% of the animals ovulated, the luteal phase length was 9.4 days, and the mean interval from calving to ovulation was 21.0 days. In conclusion, high-production dairy cows have a low frequency of ovarian activity (17.6%) and a smaller DF diameter (9.2mm) at d5pp compared to other days ( $P<0.05$ ). A significant increase in animals showing ovarian cyclicity occurred from d10 pp until d40, and the highest percentage of animals exhibited a DF on d35. The interval from calving/pregnancy ( $R=78.6$ ) and the number of artificial inseminations/pregnancy ( $R=88.9$ ) showed a high correlation with the pregnancy rate.*

**Keywords---** Puerperium, Follicular Dynamics, Ultrasonography, Dairy Cow

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## 1. INTRODUCTION

The reproductive process in the puerperal period is based on the return of the uterus to its physiological state and ovarian activity. In cattle, this process is regulated by variables such as age, parity, reproductive season, milk production [33], nutrition, changes in weight, the body condition score (BCS), and the resumption of ovarian activity [4]. [33] found a positive correlation between ovarian activity and the time of full uterine involution. Uterine involution was completed significantly earlier in cows in which ovarian activity was detected before day 30 (d30) postpartum (pp) compared to cows in which ovarian activity was detected later. [14], who tracked the puerperium period weekly in dairy cow until d72, reported that cows that had ovulated before d30 pp conceived earlier than those that had ovulated later.

[20] reported that animals in puerperium under poor conditions had a dominant follicle (DF) that produced insufficient amounts of estradiol (E2), which inhibited the induction of gonadotropin-releasing hormone (GnRH) release, the emergence of pre-ovulatory luteinizing hormone (LH)/follicle-stimulating hormone (FSH), and ovulation, consequently delaying the first ovulation. These changes have adverse effects on follicular steroidogenesis, follicular dynamics, and concentrations of FSH and inhibin, which will negatively interfere with uterine involution.

Follicular dynamics during the pp period are influenced by several factors. The BCS is one of the most influential factors on follicular development [4]. The reduction of BCS during puerperium affects the length of the pp anestrus [21] because of a reduction in the development and functional competence of the follicle [6]. An energetic deficit in the early pp

period in high-production dairy cows can repress ovarian activity, suppressing the release of gonadotropins required for the growth of the ovarian follicles and altering the dimensions of the pre-ovulatory follicle [3].

The early return of pp ovarian activity is directly related to the first service [14]. The sooner that follicular dynamics begin, the earlier estrus will occur, which makes the first artificial insemination (AI) possible. Slow ovarian activity in puerperium is the main limiting factor for the success of subsequent reproductive management programs that are directed towards AI [28].

Given the importance of the pp course on the ovarian activity and fertility of high-production dairy cows, the present study aimed to track the physiological parameters of ovarian follicular dynamics by ultrasonographic examinations of the ovaries every five days after calving. In addition, other reproductive and productive parameters were assessed for their correlation with the pregnancy rate.

## **2. MATERIALS AND METHODS**

We studied 34 black and white Holstein cows that did not show signs of complications during the peri-partal period and puerperium. The animals were maintained by using the free-stall system with non-masonry walls. The average milk production was 34.9 L/day for two milking sessions, and the body condition score (BCS) was 3.6 (1=thin, 5=obese) [17]. The feed consisted of corn silage (28% DM), barley residue (21% DM), tifton (87.6% DM), feed VL-19 (88.8% DM), soybean meal (87.5% DM), ground corn (88% DM), protected fat (95% DM), sodium bicarbonate (99%), mineral salt (99% DM), and limestone (99% DM), and the consumption of these components in kg [DM/(cow·day)] was 10.62, 2.10, 0.64, 7.99, 2.63, 0.88, 0.29, 0.20, 0.15, and 0.10, respectively.

## **3. ULTRASONOGRAPHIC EXAMINATION OF THE OVARIES**

Ultrasonographic examinations (UE) of the ovaries were carried out at 5, 10, 15, 20, 25, 30, 35, and 40 days after calving [24]. The following parameters were observed: diameter of the DF (DF $\geq$ 8 mm in diameter; [7], the second largest follicle (subordinate follicle), and the diameters of the OF and corpus luteum. Moreover, we assessed the proportion of animals undergoing ovulation, the luteal phase length, the proportion of cows with a DF after d5 pp, the BCS, parity, milk production, the interval between calvings, the number of AI/pregnancy, the overall pregnancy rate, and the intervals from calving to the first ovulation, from calving to the first AI, and from calving to pregnancy. Ultrasonography (US) of the ovaries was performed by using the transrectal approach with the AlokaSSD500-Fujihira Industry Co.Ltd.(Tokyo,Japan) 5-MHz transducer.

## **4. STATISTICAL ANALYSIS**

The means were compared by using the PROCGLM (SAS 1996, Cary, NC). The correlations between the productive and reproductive variables versus ovarian follicular dynamics were assessed using the PROC CORR procedure of SAS<sup>TM</sup> (Cary, NC, 1996) altogether with all observations, as well as for each individual variable. The pregnancy rate was correlated with all other variables by using the PROC CORR of SAS<sup>TM</sup> (Cary, NC, 1996). (Table 2)

## **5. RESULTS AND DISCUSSION**

The variables were assessed for their correlation with the pregnancy rate. Among the variables correlated with the pregnancy rate (66.6%), correlations were observed between the interval from calving to pregnancy (days) (R =78.6) and the number of inseminations/pregnancy (R =88.9). The other variables did not show correlations.

Ovarian follicular dynamics cease at approximately 21 days leading up to calving [7] and restart in the first days after birth [7,30,27]. A major objective of this study was to track ovarian activity during the pp period. Although the uterus becomes large during the first days after calving and hinders the ovaries touch [14], we observed different follicle sizes. On d5 pp, only 17.6% of the animals had a DF (9.2-mm diameter) or SF (4.9-mm diameter) (Table 1). [27] reported similar findings in dairy cows. In cows with out complications at calving and in physiological puerperium, we expected greater recruitment of ovarian follicles as a result of a sequential increase in FSH concentrations [2]. This action of the first wave of FSH would lead to the appearance of DFs in the first five days after parturition, and this was confirmed in the present study in a low percentage of cows. However, a significant increase (P<0.05) in the percentage of animals with a DF (76.4%) and a DF diameter of 13.9mm was verified at d10 pp compared to d5 (Table 1). [26, 19], who tracked follicular dynamics in cows, detected DFs (>8.0mm) between d14 and d28 and between d10 and d14 pp, respectively, which are both later than the findings in the present study and therefore highlight the difficulties of re-establishing ovarian activity in early puerperium. The data variation at d5 observed in the present study could be due to the occurrence of the significant metabolic changes associated with parturition and early lactation [10], which is more pronounced in cows without a DF until d5 pp.

Table1– Ovarian parameters tracked byultrasound examinations from day5 till d40 postpartum (pp) in Black and White Holstein cows at intervals of 5days one another (n=34)

Variables	Days								
	5	10	15	20	25	30	35	40	
Ø OF(mm) (x±s)	21,0±1,0								
Calving/1st ovulation (days) (x±s)	21,7±7,2								
Ovulation till d 40 p.p.(%)	67,7								
Ø CL (mm) (x±s)	23,3±2,2								
Lenght luteal phase (days) (x±s)	9,4±2,8								
Ø DF(mm) (x±s)	9,2±1,0 <sub>a</sub>	13,9± 4,8 <sup>b</sup>	14,6±4,5 <sup>bc</sup>	16,0±6,7 <sup>bc</sup>	15,7±5,9 <sup>bc</sup>	17,0±5,9 <sup>c</sup>	16,5±5,2 <sup>c</sup>	15,8±3,2 <sup>bc</sup>	
Ø SF (mm) (x±s)	4,9±1,1	7,6±3,0	9,7±3,5	9,1±3,9	9,4±3,5	10,5±4,2	10,4±2,9	10,8±2,9	
Cows with DF (%)	17,6 <sup>a</sup> (6/34)	76,4 <sup>b</sup> (26/34)	82,3 <sup>bc</sup> (28/34)	88,2 <sup>bc</sup> (30/34)	91,1 <sup>bc</sup> (31/34)	94,1 <sup>c</sup> (32/34)	97,0 <sup>c</sup> (33/34)	82,3 <sup>bc</sup> (28/34)	

OF= Ovulatory follicle; DF= Dominant Follicle; FS= Subordinate Follicle

Different letters on the same row indicate significant difference at P<0.05

Table 2 - Productive and reproductive variables on high production Black and White Holstein cows along the physiologic puerperium until d 40 (n = 34).

BCS (1-5)	Parity (n)	Intervall Calving /ovulation (days)	Intervall between calvings (days)	Intervall calvings/1 <sup>st</sup> AI (days)	Intervall calvings/pr egnancy(da ys)	AI/pregn ancy (n)	Pregnancy rate (%)	
3,6±0,2	3,1±1,6	34,9±5,0	21,0±7,0	401,0±38,5	83,4±11,4	140,3±52,0 <sub>a</sub>	3,5±1,9 <sup>b</sup>	66,6 <sup>c</sup>

Variablesthat showedsignificantcorrelation: <sup>a</sup> versus<sup>c</sup>(R =78.6) <sup>b</sup>versus<sup>c</sup>(R =88.9).

The data regarding the first pp ovulation are discrepant between different studies, and this is presumably because of variations in breed, production, nutrition, management, the course of parturition, and the dimensions of the new born calf, among other causes. The mean interval between calving and the first pp ovulation in this study (21.0 days) (Table 1) was higher than that reported by [27] (17.3days) and was smaller than that reported by [33] (25 days), [16] (28 days), [18] (32 days), and [1] (39.3days). However, our interval was consistent with those reported by [11,12]. The observation of different intervals between calving and the first ovulation [33] is positively correlated with the weight loss of the

parturient and the period of full uterine involution and is inversely correlated with the reduction of total digestible nutrients (TDN) and the levels of crude protein. According to [20], the BCS, age, month of calving, and disease are factors that greatly influence ovulation in the first pp DF. Cows that have a poor body condition (<2.5) during calving are more likely to be affected by anestrus because they have a low frequency of LH pulses and a subsequent reduction in estradiol concentrations to levels that are insufficient to induce the LH surge and ovulation. Cows that have a poor BCS after calving will have a decrease in the DF diameter, reduction in the concentrations of insulin and IGF-1, and low-frequency LH pulses.

The mean diameter of the OF this study (21.0 mm) (Table 1) was higher than that reported by [27] (17.6 mm). This difference can be attributed to the genetic quality of the animals, the feed, or to the mean BCS of 3.6 of the cows in this study, which was higher than that of the animals used by the cited authors ( $\geq 2.5$ ). [8,9,13,31,15,25] reported different OF dimensions in other cow breeds when the cows were not in puerperium (13.1, 15.3, 16.0, 16.5, 18.2, and 15.3 mm, respectively). According to [5], cows with low concentrations of progesterone (P4) tend to have a larger OF diameter (20.4 mm) than cows with high P4 concentrations (OF=17.2 mm), and animals with lower P4 have a higher LH concentration than cows with high P4 (0.98 vs. 0.84 ng/mL). This suggests that the amount of plasmatic P4 differs between puerperal animals and animals outside of the pp period; thus, puerperal animals have a higher concentration of LH and consequently have a greater OF diameter. In the present study, the DF diameters were smaller in the first two weeks of puerperium compared to the following weeks.

The large differences in the DF diameters between d5 and d40 pp observed in the present study could be explained by the gradual adaptation of the cows to the normal metabolic changes of this critical period, which limits the mobilization of tissues and reduces the catabolic state. These changes lead to stabilization of the metabolic state in the animals, with subsequent increases in the concentrations of glucose, insulin, IGF-1, and gonadotropins, which favor follicular development. As a new follicular wave and a period of follicular dominance begin in the animals [7,31], some follicles can also become a factor that could explain the difference between the DF diameters.

The mean diameter of the first pp corpus luteum (CL) (23.3 mm; Table 1) in our study was very similar to that reported by [31] (23.7 mm), but was lower than that reported by [15] (30.7 mm) in crossbred cows outside of the puerperium period. These results suggest that the development of the CL in the pp period is similar to that of the CL outside of the pp period. [32] reported that a low quantity of LH is stored in the pituitary after calving. We hypothesized that animals calving in good body condition and receiving proper nutrition have concentrations of LH and IGF-1 [7] that are sufficient for effective luteinization and CL formation. The average CL diameter in this study (23.3 mm) was higher than the OF diameter (21.0 mm), which corroborates the reports of [29] and [22], and indicates that nursing cows that ovulate larger follicles tend to have a larger CL because of the positive correlation between the OF size and the luteal tissue volume.

The luteal phase observed in this study (9.4 days) was shorter than the estrous cycles observed outside of puerperium. This finding is consistent with those of [14,20], who stated that the pp luteal phase is shorter because of the premature release of PGF2 followed by the increased production of estradiol by the formation of a post-ovulatory DF.

The correlation between productive and reproductive variables and the pregnancy rate (Table 2) were assessed by statistical analysis. As anticipated, a correlation between the interval between parturition and pregnancy and the number of AIs per pregnancy was verified. The other variables were not correlated with the pregnancy rate according to the data obtained in this study.

## 6. CONCLUSIONS

The ovarian activity in dairy cows was significantly lower on d5 after calving, with a smaller proportion of animals exhibiting a DF and the observed DFs had a smaller diameter relative to other days. A significant increase in cycling cows was observed from d10 pp, and this increase continued until the end of the study. The highest proportion of animals with a larger DF occurred on d35, whereas the largest average DF diameter was found on d30 pp. Only the interval from calving to pregnancy and the number of AI/pregnancy showed a high correlation with the pregnancy rate.

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