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Follicular and luteal morphometry, progesterone concentration and early gestation in Holstein cows (Bos Taurus) at high altitude in the tropics (Colombia)

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Original

Follicular and luteal morphometry, progesterone concentration and early gestation in Holstein cows (Bos Taurus) at high altitude in the tropics (Colombia)

Morfometría folicular y luteal, concentración de progesterona y éxito de la gestación en vacas Holstein (Bos Taurus) en el trópico alto (Colombia)

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Abstract:

Objectives. The aim of this study is to evaluate the relationship among ovulatory follicle (OF) size, corpus luteum (CL) volume, and progesterone (P4) serum levels with pregnancy success. **Materials and methods.** Nineteen Holstein cows located in Bogotá, Colombia with a history of 2 to 6 previous calving were used in the study. Following 40 day postpartum, two consecutive phases were evaluated as follows: 1) estrous cycle; 2) early pregnancy or return to estrus after Artificial Insemination (AI). Ultrasound exam was performed twice daily after first sign of estrus to evaluate OF diameter and CL volume, and serum P4 levels were evaluated on days 6, 9, 12 and 15 after ovulation. Cows were inseminated 12 hours post estrus signs and pregnancy was diagnosed 30 days post AI. **Results.** 47.36% of the cows were diagnosed as pregnant after AI. Pregnant cows had smaller OF diameters (17.85±2.39 mm) than non-pregnant females (21.10±2.86 mm) ($p<0.05$). Cows with smaller OF were more likely to become pregnant (OR=0.624, IC=95% (0.4-0.9) ($p<0.05$). There were non significant differences in the CL volume ($p=0.10$) and P4 serum values ($p=0.39$) between pregnant and no pregnant cows on days 6, 9, 12 and 15 post ovulation. Serum P4 levels were not correlated to CL size and reproductive status. **Conclusions.** This study shows that there was not significant difference in serum P4 levels and the CL volume when comparing pregnant with non-pregnant cows. A relationship was found between the OF diameter and the CL volume. Small OF diameter was a factor associated with early pregnancy.

Keywords: Estrous cycle, follicular dynamics, ovary, postpartum, pregnancy, reproduction.

Resumen:

Objetivo. Evaluar la relación entre el diámetro del folículo ovulatorio (FO), el volumen del cuerpo lúteo (CL), los niveles séricos de progesterona (P4) con el éxito de la gestación. **Materiales y métodos**. En 19 vacas Holstein que habían tenido 2 a 6 partos ubicadas en Bogotá, se evaluaron dos fases consecutivas a partir del día 40 postparto, la primera correspondió al ciclo estral y la segunda correspondió a la gestación temprana o repetición de celo. En cada fase se detectó el celo, se realizó ultrasonografía para evaluar el diámetro del FO, confirmar ovulación, y el volumen del CL los días 6, 9, 12 y 15; en estos días también se evaluó los niveles séricos de P4. En la segunda fase se realizó inseminación artificial (IA) y se diagnosticó la gestación 30 días post-IA. **Resultados.** El 47.36% de las vacas se diagnosticaron como gestantes y el 52.63% como no gestantes. Las gestantes presentaron diámetros de FO menores 17.85±2.39 mm al de las no gestantes (21.10±2.86 mm; $p<0.05$). Las vacas que presentaron diámetros de FO más pequeños tuvieron mayor posibilidad de quedar gestantes (OR=0.624, IC=95% (0.4-0.9) ($p<0.05$). Los animales gestantes y no gestantes presentaron similares volúmenes de CL ($p=0.10$) y niveles séricos de P4 ($p=0.39$) los días 6, 9, 12 y 15. **Conclusiones.** No hubo diferencias en los niveles séricos de p4 y el volúmen del CL. Vacas con FO de menor diámetro tuvieron mayor probabilidad de gestación. Otras variables como CL y niveles de P4 no fueron predictivos del éxito de la gestación.

Palabras clave: Ciclo estral, dinámica folicular, ovario, postparto, preñez, reproducción.

INTRODUCTION

In ruminants, a successful pregnancy depends on complex interactions between the mother and the conceptus in the genital tract of the female (1). During maternal pregnancy recognition, the viable embryo must be able to interrupt the luteolysis with the production of Bovine trophoblastic interferon, and promoting the action of a functional CL that synthesizes adequate levels of P4 (2), a hormone that plays a vital role in early gestation, since it stimulates and maintains the functions necessary for the growth of the conceptus, implantation and placentation (3,4).

An OF with appropriate size can become, after ovulation, a functional CL that will secrete P4 with a positive effect on the establishment of pregnancy (5,6). Although the results from different studies have been variable and contradictory (7,8,9,10). Other studies suggest that there is no association of the diameter of the ovulatory follicle, size of the CL, serum levels of P4 with pregnancy rates (11,12).

Given the wide variation in the abovementioned results, it was considered worth to evaluate OF diameter, CL volume, serum progesterone levels and their possible relationship with the estrous cycle and success of pregnancy, under the specific environmental conditions in the Sabana of Bogotá (Colombia), at an altitude of 2565 meters above sea level, average temperature of 13ºC and relative humidity of 80-85%.

MATERIALS AND METHODS

Location and animals. The study was conducted at the Marengo Agricultural Center of the Universidad Nacional de Colombia, located Bogotá-Mosquera, with an altitude of 2565 meters above sea level. Average annual temperature of 13°C, with fluctuations between 4°C and 20°C, a relative humidity of 80% to 85% (13).

Nineteen Holstein cows with a history of 2 to 6 previous calving and a body condition >2.5 (1=thin, 5=obese), were used in the study, under a rotational grazing system. The cows are maintained under appropriate welfare conditions as a part of a herd health program.

Cows were found with good sanitary conditions, with the current vaccination plan (Brucellosis and foot-and-mouth disease). At the beginning of the experiment, only the females that did not present clinical reproductive pathologies such as metritis, endometritis, follicular or luteal cysts among others, were selected; which was determined through visual inspection of the external reproductive organs, rectal palpation, ultrasonography and vaginoscopy.

Cows enrolled in the study were evaluated starting 10 days postpartum. Rectal palpations and trans-rectal ultrasound were performed in order to determine return to cyclicity, absence of reproductive pathologies and the occurrence of one regular estrus cycle. From 40 days post-partum on, only cows which completed the abovementioned conditions were selected (14), they were enrolled in the study. Heat detection, rectal palpations, ultrasound, and blood sampling, to evaluate serum levels of P4, were performed as explained below.

Estrus Detection. The detection of estrus was achieved by visual observation performed four times a day by the same persona throughout the experiment. The observation periods were as follow, from 6:00 to 7:00 am, 12:00(noon) to 1:00 pm, 5:30 to 6:30 pm and from 9:00 to 10:00 pm. The beginning of estrus was determined when a cow showed restlessness, attempt to mount other females or permit them to do so, licking and sniffing of external genitals, or the presence of vulvar mucus; estrus was determined when a cow repeatedly allowed to be mounted by other females (15).

Rectal Palpation and Trans Rectal Ultrasound examination. Ultrasound was performed by an expert professional throughout the experiment with a Pie-medical Aquila Pro Vet ultrasound scanner, equipped with a 7.5 MHz linear trans rectal transducer.

From the 40th postpartum day on, two consecutive phases were evaluated, the first phase corresponded to the first estrous cycle and was defined as the lapse between the presentation of the first and second estrus (Day 0), following the latter all the cows enrolled were inseminated. The second phase corresponded to early gestation or return to estrus starting on Day 0. Pregnancy diagnosis was performed on day 30 (Figure 1).

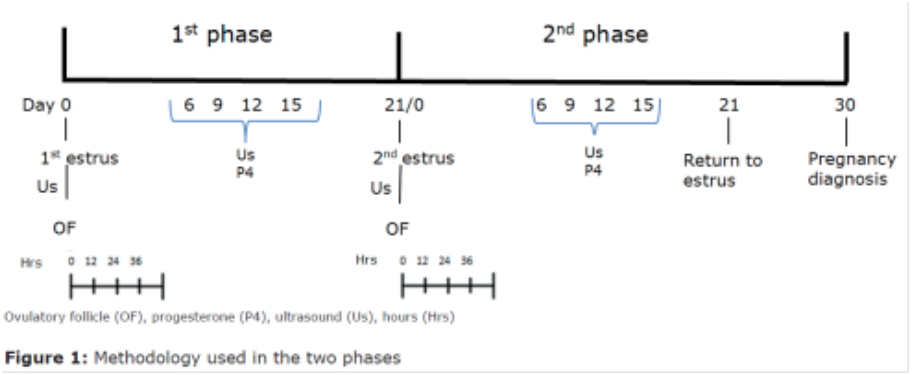

FIGURE 1
Figure 1

Ultrasound examination was performed at the first signs of estrus and subsequently repeated every 12 hours until ovulation was confirmed defined as the OF disappearance and the subsequent appearance of a CL. The largest diameter of the OF was recorded for analysis (Figure 1).

Additionally, ultrasound was performed on days 6, 9, 12 and 15 of each phase in order to evaluate the diameter of the CL in mm. Cl Volume was calculated with a formula previously reported (11) as follows, $4/3 \times \pi \times r^3$, in which the value of π (3.1416) is a constant and r corresponds to the radius (diameter divided by 2) of the size of the CL. For CL with a cavity, the volume was calculated with the same formula and the volume of the cavity was subtracted from volume of the luteal tissue. A functional CL was considered as such when serum levels of P4 on day 6 after estrus were greater than 1 ng/ml (11).

Pregnancy diagnosis was performed on day 30 post AI. A cow was considered pregnant when there was presence of amniotic fluid and an embryo with a heartbeat (11).

Blood samples and hormonal analysis. The serum P4 levels of days 6, 9, 12 and 15 of the two consecutive phases were analyzed. Subsequently, ELISA technique with a "DS-EIA-Steroid-progesterone" kit, RH-351, (Italy) was used for the quantification of serum P4 levels. The serum levels of P4 were adapted to the test by standardizing the curve, taking into account what was reported by the literature in bovines (14). The test had a lower sensitivity limit of 0.1 ng / ml and an inter-assay and intra-assay variation coefficient of 4.2% and 3.6% respectively.

Statistical analysis. For the statistical analyzes of the two consecutive phases, data was normalized to the estrus manifestation day (day 0), and for days 6, 9, 12 and 15 of each phase. The information obtained from each of the variables was initially analyzed using descriptive statistics with mean values and standard deviation (SD). Animals with 2 and 3 follicular growth waves were compared using the Student's t test (16).

Subsequently, a general linear model of repeated measures was used, in which the repeated measurements were on days 6, 9, 12 and 15, and the variables were: serum levels of P4 and CL volume. The presence of significant differences among the levels were analyzed with the orthogonal polynomial test and normality was evaluated according to Scheffe' test (16).

The relationship among independent continuous variables: volume of CL, OF diameter, interval from the beginning of estrus to ovulation, interval from the beginning of estrus to AI, P4 serum levels in relation to independent variable reproductive status (pregnant, non-pregnant on day 30 after the AI), was determined by binary logistic regression. The strength of the association was estimated through the odds ratio (OR) calculation. To determine the relation between OF diameter, luteal volume and serum levels of P4 in each

cycle, linear regression analysis was used. All $p<0.05$ were considered significant. The statistical software used was SAS 9.3 program (SAS 2002 Institute, Cary, NC, USA) and SPSS® version 22.

RESULTS

Follow-up of the first phase: estrous cycle. On average, first heat was observed on day 61.36±26.7 postpartum and the duration of the correspondent cycle was 22.94±1.8 days (mean ± SD) with a range between 21 to 26 days. 47.36% (9/19) of the animals presented 2 waves of follicular growth, of which 66.6% (6/9) had an estrous cycle duration of 21 days and 33.3% (3/9) of 22 days. Cows with 3 follicular waves (52.63% (10/19)) had estrous cycle duration of 23 days (30% (3/10)), 24 days (20% (2/10)), 25 days (30% (3/10)) of and 26 days (20% (2/10)) (Figure 2).

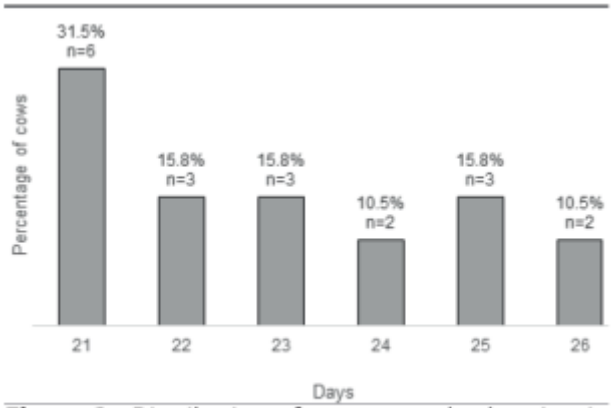

Figure 2. Distribution of estrous cycle duration in postpartum Holstein cows.

FIGURE 2
Figure 2

There were no significant differences in the OF diameter among animals that presented 2 follicular waves and those of 3 follicular waves ($p>0.05$, Table 1). The time elapsed between the beginning of estrus and ovulation was on average of 27.66±6.32 h. for cows with 2 follicular waves and of 28±6 h. for cows with 3 waves ($p>0.05$), with a range between 21 at 36 h.

After ovulation, ultrasound evaluation of luteal growth was performed. Days 12 and 15 had the largest size of CL during the study period. CL on Day 12 had an average volume of 13800±2788.72 mm^3 in cows with 2 follicular waves and of 16343.88 ± 3579.05 mm^3 on cows with 3 waves. Similarly, on day 15, an average luteal volume of 13814.98 ± 2655.17 mm^3 was observed in cows of 2 waves and of 16342.83±3542.1 mm^3 in those with 3 waves. There were no significant differences.

Serum levels of P4 for all the cows on day 6 of the estrous cycle were >3 ng/ml (4 ±0.9 ng/ml). On day 12 the highest levels were found; cows with 2 follicular waves had 6.43 ± 0.46 ng/ml of P4 and cows with 3 follicular waves had 6.52±0.32 ng/ml (Table 1, Figure 3).

TABLE 1.
Table 1. Comparison between animals with 2 and 3 follicular waves in the first cycle for values (average ± SD) for OF diameter (mm), CL volume (mm^3), serum P4 concentration (ng / ml) and time elapsed from the beginning of estrus to ovulation.

Variable	2 Waves	3 Waves	P-value
OF (mm)	19.87 ±2.8	19.83 ±2.8	0.97
Time elapsed from the beginning of estrus to ovulation (h)	27.66 ±6.3	28.00 ±6.0	0.91
P4 (ng/ml) day 6	4.30 ±0.9	4.97 ±0.8	0.13
day 9	6.07 ±0.5	6.08 ±0.6	0.96
day 12	6.43 ±0.4	6.52 ±0.3	0.65
day 15	6.53 ±0.3	6.54 ±0.3	0.92
CL Volume (mm^3) day 6	7766.89 ±1985.4	8902.86 ±2863.5	0.34
day 9	11629.68 ±2527.6	14736.05 ±4225.1	0.07
day 12	13800.08 ±2788.7	16343.88 ±3579.1	0.11
day 15	13814.98 ±2655.2	16342.83 ±3542.1	0.10
Ovulatory Follicle (OF), Corpus Luteum (CL), progesterone (P4) *Values p<0.05 were considered as statistically significant. ** The reported values correspond to the average ± ED			

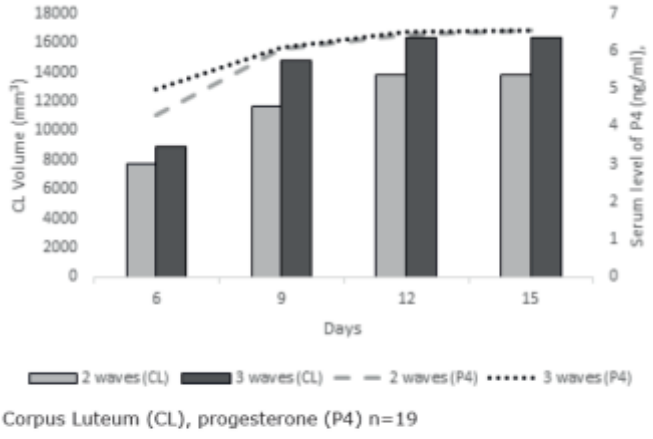

Figure 3. Progesterone serum concentrations and corpus luteum volume on days 6, 9, 12 and 15 of the 1st estrous cycle in Holstein cows with 2 and 3 follicular growth waves.

FIGURE 3
Figure 3

Follow-up of the second phase: Pregnant and non-pregnant cows. The second cycle began when the second heat was detected at which point, all the cows in the study were inseminated 12.8 ± 1.8 h after the estrus signs where first seen. On average, cows where AI at 89.4 ± 34.7 days postpartum; with an average milk production of 24.4 ± 4.9 Lts/day (range of 16.4 to 32.7 Lts). After ovulation, ultrasound evaluation of luteal growth was performed on days 6, 9, 12 and 15. Thirty days post-AI, 47.36% of the cows were diagnosed as pregnant (9/19), and 52.63% (10/19) as non-pregnant by trans-rectal ultrasound examination.

The OF from pregnant cows had significantly ($p<0.05$) smaller diameter (17.85±2.39 mm) when compared with non-pregnant cows (21.10±2.86 mm) as seen in Table 2.

TABLE 2

Table 2. Comparison of the values (average ± standard deviation) of OF diameter (mm), CL volume (mm^3), serum P4 concentration (ng / ml), time elapsed from the beginning of estrus to ovulation in pregnant and non-pregnant cows.

Variable	Pregnant Average ± DE	Not pregnant Average ± DE	P-value
OF (mm)	17.85 ±2.3	21.10 ±2.8	0.01*
Time elapsed from the beginning of estrus to ovulation (h)	30.00 ±7.3	28.00 ±6.0	0.53
Time from AI to ovulation (h)	17.33 ±6.3	16.00 ±6.0	0.65
P4 (ng/ml) day 6	4.08 ±1.1	4.51 ±0.6	0.35
day 9	6.05 ±0.4	6.21 ±0.4	0.47
day 12	6.48 ±0.3	6.58 ±0.4	0.62
day 15	6.65 ±0.3	6.67 ±0.4	0.93
CL Volume (mm^3) day 6	7325.57 ±1891.6	7207.45 ±1978.7	0.89
day 9	10353.88 ±2688.7	11926.27 ±2200.1	0.19
day 12	12487.43 ±2988.2	15518.63 ±3656.0	0.07
day 15	12524.88 ±2959.5	14935.41 ±3599.4	0.14
Ovulatory Follicle (OF), Corpus Luteum (CL), progesterone (P4) *Values p<0.05 were considered as statistically significant. ** The reported values correspond to the average ± ED			

No significant differences were found in P4 levels in pregnant and non-pregnant cows during the studied days. On day 6 of the cycle P4 levels were on average 4.08±1.1 ng/ml for the group of pregnant cows, and 4.51 ± 0.6 ng / ml for non-pregnant. The highest serum levels of P4 were found on the day 12; 6.48±0.3 ng/ml for pregnant cows, and 6.58±0.4 ng/ml for non-pregnant animals. Similarly, on day 15, P4 mean levels found in pregnant animals were 6.65±0.3 ng/ml and 6.67 ± 0.4 ng/ml in non-pregnant animals (Table 2, Figure 4).

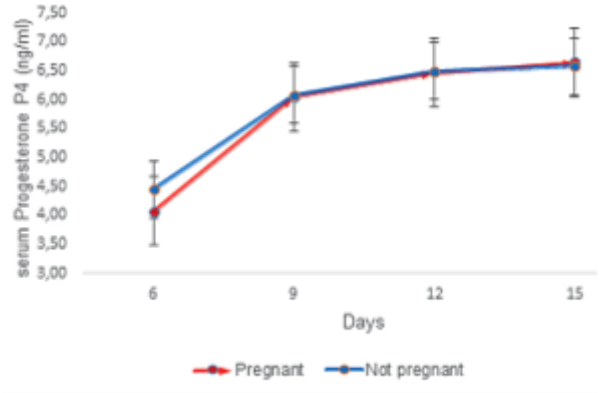

Figure 4. Serum levels of Progesterone (P4) (ng/ml) in pregnant and non-pregnant cows

FIGURE 4
Figure 4

Similarly, there were no significant differences in luteal volume in pregnant and non-pregnant cows (p=0.10), (Table 2, Figure 5); Regardless of size and reproductive status, CL produced similar serum levels of P4.

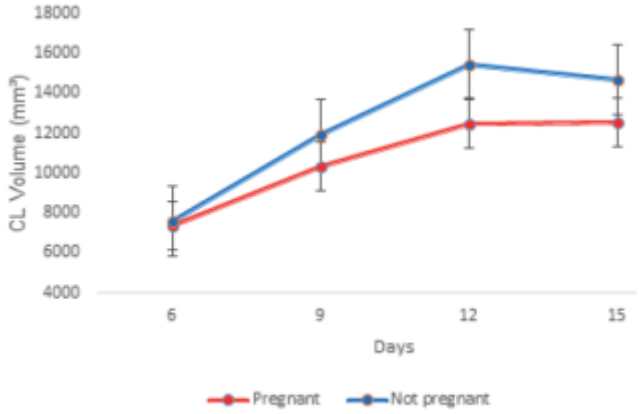

Figure 5. Volume of Corpus luteum (CL) (mm³) in pregnant and non-pregnant cows.

FIGURE 5
Figure 5

However, when evaluating non-pregnant cows (10/19), females with normal return to estrus (5/10) and others with late return (5/10) were found. The females to normal return to estrus were those that had a cycle length in the second phase (22.6±1.5 days) similar to the first phase (23±1.4 days). Cows considered with a delayed return to estrus where those that had a greater cycle length (33.4±7.7 days) compared with their first phase (23.6±2.7) (Table 3).

TABLE 3.
Table 3. Comparison between OF diameter and duration of the first and second phase in cows of normal and late return to heat.

Reproductive status	Variable	First phase	Second phase	P-value
Normal return to estrus	Duration (days)	23±1.4	22.6±1.5	0.47
	OF (mm)	21.38±2.6	19.72±2.7	0.08
Delay return to estrus	Duration (days)	23.6±2.7	33.4±7.7	0.04
	OF (mm)	19.74±2	22.46±2.02	0.02

In addition, females with normal return to estrus had smaller OF diameters in the second phase (phase in which AI was performed) (19.72±2.76mm) compared to the first phase (21.38±2.6mm). In contrast to these results, the cows with late return to estrus had higher OF diameters in the second phase (22.46±2.02mm) compared with the first (19.74±2mm) (Table 3).

Association between serum levels of P4, CL volume, and the diameter of the OF

After comparing pregnant and non-pregnant animals regarding the OF diameter, volume of CL and serum levels of P4, the relationship between these variables was evaluated. A linear relationship was found between the OF diameter and the CL volume ($p<0.05$). Additionally, in the first phase there was a linear relationship between CL volume and serum P4 levels ($p<0.05$), however in the second phase this relationship was not found ($p>0.05$).

On the other hand, the repeated measures model showed that the mean values of the CL volumes and the serum levels of P4 were significantly different ($p<0.001$) along the time period studied (days 6, 9, 12 and 15) and this relationship was linear ($p<0.001$) and quadratic ($p<0.001$).

Association between OF diameter, CL volume, serum P4 levels, and early pregnancy. When evaluating the association between OF diameter, CL volume, P4 and pregnancy, only small OF diameter (18.1 mm) was a factor associated with early pregnancy (OR=1.65, 95% CI (1.039-2.619) ($p<0.05$)(Table 4). That is to say that the smaller diameter of the OF, the greater the probability of gestation.

TABLE 4
Table 4. Association between the OF diameter, Corpus Luteum (CL) volume and serum levels of progesterone (P4) during the second phase and gestation at day 30 post-AI.

Variable	OR	95% CI	P-value
OF	1.65	1.039-2.619	0.034
CL day 6	1.0	1.000-1.001	0.769
CL day 9	0.5	0.23-13.801	0.72
CL day 12	5.8	0.25-13.678	0.52
CL day 15	0.06	0.23-10.862	0.30
P4 day 6	1.62	0.543-4.822	0.38
P4 day 9	1.0	1.000-1.001	0.19
P4 day 12	0.99	0.997-1.001	0.33
P4 day 15	1.0	0.99-1.004	0.20

DISCUSSION

In the present study, we found cows with 2 and 3 follicular growth waves during an estrous cycle as reported in studies conducted in other countries (17,18,19).

When comparing animals with 2 and 3 follicular waves; there were no significant differences in OF diameter, with both groups of animals having an average diameter of 19.8 mm. It is possibly that no significant differences were found because they presented similar growth rates and duration of the ovulatory wave. These results differ from those of other authors (20), who reported differences in the diameter of the OF between cows with 2 and 3 follicular waves. Cows with 2 follicular waves and those with 3 follicular waves had an average diameter of 15.03 mm and 14.68 mm respectively. Our results may be an incidental finding and require further investigations to determine if there are differences in the diameter of the OF between cows with 2 waves and those with 3 waves and if there are different factors that can influence the presentation of a certain pattern of follicular growth in females.

In this group of animals, it was also observed that there were no significant differences in the average time elapsed from the beginning of estrus to ovulation between cows with 2 follicular waves (of 27 hours) and those with 3 waves (28 hours), in agreement with reports indicating that physiologically, ovulation occurs between 25 and 36 hours after the end of estrus (11).

On the other hand, the volume of the CL and the serum levels of P4 on days 6, 9, 12 and 15 were similar in the animals with 2 and 3 waves of follicular growth. This is probably given to the fact that around day 6 post ovulation there is a functional CL producing serum levels of P4 greater than 1 ng/ml regardless of the number of waves.

Most likely, significant differences would be seen when comparing the beginning of luteal regression and the decline in the serum levels of P4 as cows with 2 waves initiate this process before cows with 3 waves. Furthermore, several authors have found no significant differences in the maximum diameter of CL and the highest serum levels of P4 between 2 and 3 waves cows (20,21,22,23,24).

In the second phase of the present study, there were no significant differences ($p>0.05$) in the interval from beginning of estrus to ovulation between those cows that became pregnant (30 hrs.) and those that returned to estrus (28 hrs.). This indicates that this interval is not a factor that can be associated with gestation in this group of animals (11).

Pregnant cows had significantly smaller OF diameters than non-pregnant cows; It was found that the small OF diameter was a factor associated with diagnosis of early gestation. It is possible that the duration of the ovulatory wave was longer in non-pregnant animals and that it took longer time to decrease the serum levels of P4, leading to an increase in the size of the dominant follicle due to a longer time of follicular development. Possibly, these large follicles took longer to reach the estrogen levels needed to trigger the estrus behavior and thus there was an anticipated stimulus in the secretion of LH that could cause early maturation or resumption of the meiosis of the oocyte before the ovulatory pick of LH. This could explain that at the time of ovulation, the fertilization ability of the ovum has been affected decreasing fertilization rate or resulting in an early embryonic death before maternal recognition.

According to Mimh and colleagues (25), when the dominance period lasted between 1 and 4 days, a high pregnancy rate was found, while dominance greater than 10 days was associated with a decrease in pregnancy. This is probably due to the fact that the increase in the production of estradiol over a long period of dominance affects the normal oocyte maturation (26) affecting fertilization or causing alterations in early embryonic development (27).

Extending the duration of follicular dominance by using a P4 device resulted in a larger OF size (11 vs 16 mm) and lower conception rate (65 vs 37%) (28). In another study (29), researchers found that OF> 20 mm in Holstein cows was a predictor of gestation loss. However, although there are several studies that conclude that there is a relationship between the OF diameter and early gestation, it is not fully understood and there

is not a clear physiological explanation. Additional studies are needed to determine the type of association of follicular diameter with gestation and why larger follicles are associated with a lower pregnancy rate.

When analysing non-pregnant cows, we found animals with normal and late return to estrus. Those with normal return to estrus, in the two phases evaluated, had the same number of follicular waves, similar length of cycles and similar average OF diameters during the 2 cycles evaluated. Fertilization may have failed or conceptus could died before pregnancy recognition.

In the case of the cows that did not conceive and also had a late return to estrus, we observed an increase in the number of follicular waives, some took up to 43 days to return to estrus and in the ultrasound 30 days post AI some of them had persistent CL and/or anechoic fluid in the uterine horns in the absence of a fetus. The presence of fluid possibly indicates early embryonic death and reabsorption taking place after maternal recognition which may have prolong the half-life of CL, maintaining the production of P4 and causing late return to heat.

On the other hand, we found that pregnant and non-pregnant cows presented similar serum levels of P4 in the first days of the cycle (days 6, 9, 12 and 15) and that these values were not significantly different ($p>0.05$). This could indicate that the serum levels of P4 in the mentioned days, did not have an influence in the success of gestation.

These results are in agreement with previous studies (30), that found no detectable differences in the expression of genes in the endometrium of pregnant and cyclic heifers on days 5, 7 and 13 post-estrus; results that indicate that for the first days of the cycle the changes that occur in the transcriptome of the endometrium are independent of the presence of a conceptus until there is pregnancy recognition. Only after the maternal recognition of pregnancy (around day 16) there are observed differences in the endometrial transcriptome between cyclic and pregnant heifers (30). In addition, there were no differences in the luteal phase of gestating and cyclic heifers and their levels of P4. Differences between these two groups were observed after maternal recognition of pregnancy when interferon tau secreted by the trophoblast prevents luteolysis triggering changes in the endocrine system that regulates endometrial gene expression.

In the present study, a linear relationship was found in the first and second phases between the OF diameter and the CL volume ($p=0.01$); perhaps, the ability of the CL to produce P4 depends on the number granulosa cells in the OF, which may be directly related to its diameter.

The larger the OF, the greater the number of granulosa cells and, consequently, the better steroidogenic potential CL (1,7,9). However, the aforementioned size depends not only on the number of granulosa cells, but also on the number of internal theca cells, the volume of the follicular antrum, the number of receptors for LH in both cell types and the blood levels of this hormone. In a previous work (31), it was found that the volume of the CL on day 7 or 14 was significantly correlated with follicular size; and the ovulation of small follicles led to the formation of small CLs. In sheep, it was demonstrated that the premature induction of ovulation with GnRH subsequently reduces the luteal size and the number of luteal cells, related to a reduction in the number of granulosa and thecal cells in the OF that was induced to ovulate prematurely (32).

Moreover, in the first phase of the present study, a linear relationship was observed between CL volume and serum P4 levels ($p=0.01$) but in the second phase this association was not found. It is possible, then, that the aforementioned relationship does not occur regularly in all animals, which would explain the contradictory findings reported in different studies (7,8,9,10). Therefore, a CL of greater volume not necessarily produces more P4. After ovulation, the number of thecal and granulosa cells producing P4, is a function of their respective number of receptors.

In summary, we found that the OF from pregnant cows had significantly smaller diameter when compared with non-pregnant cows. No significant differences were found in luteal volume and P4 levels in pregnant and non-pregnant cows during the studied days. A relationship was found between the OF diameter and the CL volume. Small OF diameter was a factor associated with early pregnancy. That is to say that the smaller diameter of the OF, the greater the probability of gestation.

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