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Estrous behavior, ovulatory follicle dynamics, and corpus luteum size in Creole cows after spontaneous or prostaglandin F2α-induced estrous¤

Comportamiento estral, dinámica folicular ovulatoria y tamaño del cuerpo lúteo en vacas Criollas después del estro natural o inducido con prostaglandina F2α

Comportamento estral, dinâmica folicular ovulatória e tamanho do corpo lúteo em vacas Crioulas após o estro espontâneo ou induzido com prostaglandina F2α

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Summary

Background: evaluation of reproductive behavior in Creole cows is important to determine several parameters, such as fertility, that show the physiologic reproductive mechanisms under different conditions. Therefore, the sequence of events such as ovulation and the subsequent formation of a corpus luteum need to be considered to establish genetic improvement programs under synchronized or naturally-occurring estrous conditions. **Objective**: to evaluate the ovarian and behavioral estrous characteristics before, during and after prostaglandin F2α-induced or naturally occurring estrous in Creole cows. **Methods:** thirty Creole cows were subjected to estrous synchronization with PGF2α and observed continuously over five days to determine estrous onset and duration. Seventeen days after synchronized estrous detection, cows were observed during seven d to detect the naturally-occurring estrous and its duration. After the onset of both types of estrous the ovaries of each cow were ultrasounded every 8 h to determine diameter of the preovulatory follicle at deviation and

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its maximum diameter prior to ovulation, as well as its growth rate, time to ovulation and maximum diameter of the later corpus luteum. **Results:** time of appearance and duration of synchronized and spontaneous estrous were similar ($p>0.1$). Diameters of the dominant follicle at deviation and prior to ovulation were similar in both types of estrous. Nevertheless, growth rate (mm/d) of the preovulatory follicle was higher ($p<0.05$) in synchronized estrous. However, time from onset of synchronized estrous to ovulation was longer ($p<0.01$). Diameter of corpus luteum 10 d after ovulation was similar in both types of estrous. Results indicate that growth rate of follicle and time from synchronized or spontaneous estrous to ovulation must be considered for estrous cycle manipulation and artificial insemination of Creole cows. **Conclusion:** a higher proportion of Creole cows show heat during nighttime, growth rate of ovulatory follicle is higher during synchronized estrous, and time from onset of synchronized estrous to ovulation is longer, when compared with spontaneous estrous.

Keywords: *follicular growth, heat, ovulation, synchronization.*

Resumen

Antecedentes: la evaluación del comportamiento reproductivo en vacas Criollas es importante para determinar varios parámetros como la fertilidad, que muestren los mecanismos fisiológicos de la reproducción bajo diferentes condiciones. Así, la secuencia de eventos como ovulación y la subsiguiente formación de cuerpo lúteo se deben considerar al implementar programas de mejoramiento genético bajo condiciones del estro sincronizado o natural. **Objetivo:** evaluar las características ováricas y conductuales de vacas Criollas antes, durante y después del estro inducido con prostaglandina F2α (PGF2α) o del estro natural. **Métodos:** treinta vacas Criollas fueron sometidas a sincronización del estro con PGF2α y observadas continuamente durante cinco días para detectar el inicio y la duración del estro. Diecisiete días después del período de detección del estro sincronizado las vacas fueron observadas durante siete días para detectar el estro natural y su duración. Después del inicio de ambos estros, los ovarios de cada vaca fueron monitoreados mediante ultrasonografía cada 8 h para determinar el diámetro del folículo preovulatorio al momento de la desviación y su máximo diámetro alcanzado antes de la ovulación, así como su tasa de crecimiento, tiempo a la ovulación y diámetro máximo del cuerpo lúteo posteriormente formado. **Resultados:** el momento de inicio y la duración del estro sincronizado y natural fueron similares ($p>0,1$). El diámetro del folículo dominante al momento de la desviación y antes de la ovulación fueron similares en los dos tipos de estro, pero la tasa de crecimiento del folículo preovulatorio (mm/d) fue mayor ($p<0,05$) en el estro sincronizado. No obstante, el tiempo del inicio del estro sincronizado a la ovulación fue mayor ($p<0,01$). El diámetro del cuerpo lúteo 10 d después de la ovulación fue similar en ambos estros. Los resultados indican que la tasa de crecimiento del folículo y el tiempo del estro -sincronizado o natural- a la ovulación deben considerarse cuando se requiere manipular el ciclo estral para inseminación artificial. **Conclusión:** una mayor proporción de vacas Criollas mostraron estro durante la noche, la tasa de crecimiento del folículo ovulatorio es mayor durante el estro sincronizado, y el tiempo del inicio del estro sincronizado con PGF2α a la ovulación es mayor que en el estro natural.

Palabras clave: *calor, crecimiento folicular, ovulación, sincronización.*

Resumo

Antecedentes: a avaliação do comportamento reprodutivo do gado Crioulo é importante para a determinação de parâmetros como a fertilidade, que indiquem claramente os mecanismos fisiológicos da reprodução em diferentes condições. Assim, a sequência de eventos como a ovulação e subsequente formação do corpo lúteo é um grupo dos fatores a considerar quando programas de melhoramento genético são implementados sob condições do estro natural ou sincronizado. **Objetivo:** avaliar as características do ovário e o comportamento estral antes, durante e depois de um estro induzido por prostaglandinas F2α (PGF2α) ou que ocorre naturalmente. **Métodos:** trinta vacas Crioulas foram submetidas à sincronização de estros com PGF2α e observadas continuamente ao longo de cinco dias para detectar o início e tempo de duração do estro. Dezessete dias após o período de detecção do estro sincronizado, as vacas foram observadas similarmente ao longo de sete dias para detectar o estro de ocorrência natural e sua duração. Depois do início de ambos os tipos de estro, os ovários de cada vaca foram monitorizados por ultrassom em intervalos de 8 h para determinar o diâmetro do folículo pré-ovulatório de desvio e o seu diâmetro máximo antes da ovulação, assim como sua taxa de crescimento, tempo da ovulação e o diâmetro máximo do último corpo lúteo. **Resultados:** a hora do dia da apresentação e a duração dos estros sincronizados e espontâneos foram semelhantes ($p>0,1$). O diâmetro do folículo dominante em desvio e antes da ovulação foi similar em ambos os tipos de estro, mas a taxa de crescimento (mm/d) do folículo pré-ovulatório foi maior ($p<0,05$) em vacas sincronizadas com um tempo maior de início do estro até a ovulação (p<0,01). O diâmetro do corpo lúteo 10 d depois da ovulação foi similar em ambos os tipos de estro. Os resultados indicam que a taxa de crescimento do folículo e o tempo do estro sincronizado ou espontâneo até a ovulação devem ser considerados quando a manipulação do ciclo estral e a inseminação artificial de vacas Crioulas são requeridos. **Conclusão:** uma maior proporção de vacas Crioulas manifestou estro durante a noite, a taxa de crescimento do folículo ovulatório é maior durante o estro sincronizado e o tempo de início do estro sincronizado com PGF2α na ovulação é maior do que no estro natural.

Palavras chave: *calor, crescimento folicular, ovulação, sincronização.*

Introduction

Genetic improvement methodologies in cattle production systems have evolved over the years from being producer-driven to consumer-driven worldwide, as consumer needs vary over time. Thus, sustainable production systems need to be tailored to account for physical, social and market conditions of each producer and consumer community. For cattle breeders this raises the question of whether they should diversify their breeding objectives or breed animals that can produce well under a wide range of environments, management system and market conditions (Bichard, 2002). Such is the case of Creole cattle, which has been able to adapt to harsh environmental conditions (de Alba, 1987).

In production systems such as Creole cow-calf, reproductive technology has direct effects on genetic improvement rates, as the use of artificial insemination and embryo transfer result in higher selection intensity, more accurate selection of animals and more accurate estimation of the breeding value across herds (Hill, 2000). Creole cattle have been raised in northern regions of Mexico and have been proven to be able to survive and reproduce under very harsh environmental conditions, which has resulted in well-adapted animals that are sold nowadays in a growing variety of markets including rodeos and organic shops. Creole is considered a highly valuable genetic resource, and as such, requires the implementation of genetic improvement programmes. However, common problems include the lack of information on basic reproductive physiology of Creole females after a naturally-occurring or a hormonally-induced estrous. Consequently, the knowledge gathered from beef or dairy breeds has been used to manage reproduction in Creoles (Rios, 2010).

The fact that Creole cattle have been raised and adapted differently may explain the poor results obtained in artificial insemination programs after estrous synchronization, with pregnancy rates seldom meeting those obtained with other bovine breeds (Zárate-Martínez *et al.,* 2006). It is important to notice that estrous has been characterized over the years in terms of its duration and mounting activity (Van Vliet and Van Eerdenburg*,* 1996). Knowledge of estrous behavior and estrous-to-ovulation interval is essential for estimating the best time to artificially inseminate, as the understanding of the factors involved in the expression of estrous and occurrence of ovulation is an obstacle limiting the success of artificial insemination (Galina *et al.,* 1996).

Although these parameters have been well characterized for the European breeds and for some Zebu breeds, little has been published on these parameters for Creole cattle. In *Bos taurus* breeds estrous behavior lasts approximately 18 h and ovulation occurs 28 to 31 h after the onset of spontaneous or synchronized estrous, while in *Bos indicus*, naturally-occurring estrous is shorter, approximately 11 h, and ovulation occurs around 25 h after the start of estrous (Pinheiro *et al.,* 1998). Furthermore, the temporal distribution of estrous and the effects of synchronization treatments on its overt expression are important aspects that need to be considered when designing strategies for controlled breeding programs, considering that both hormonally-induced and naturally-occurring estrous may differ among different types and breeds of cattle (Larson and Ball*,* 1992; Zárate-Martínez *et al.,* 2006).

The hypothesis for the present study was that, in Creole cows, mechanisms involved with spontaneous and synchronized estrous differ. Therefore, the objective was to evaluate the ovarian characteristics and behavioral estrous occurrence and duration after prostaglandin-induced or naturally-occurring estrous in creole cows.

Materials and methods

Ethical considerations

All procedures involving animals were conducted within the guidelines of official techniques of animal care and health in México (Ley federal de sanidad animal; articles 19 to 22) and NOM-051-ZOO-1995: Humanitarian care of animals during mobilization.

Location and animals

The experiment was conducted in Ahumada, Mexico (latitude 30º 29' N, longitude 106º 28' W) during winter/springtime (last three weeks of March), under maximum and minimum environmental temperature of 23 and 10 ºC, respectively. Thirty multiparous (parity one to three) non-lactating Creole cows (*Bos taurus*) with ≥ 7 months without pregnancy body weight (BW) = 357 to 465, mean 384.4 ± 23.9 kg; body corporal score (BCS) = 4 to 5 in a scale of 0 = thin to 9 = obese, were used. All cows were identified as cyclical. Cyclicity was determined by observation of a corpus luteum (CL) 12 d before or on d of onset of the experiment via transrectal ultrasound images (Figure 1). Cows were also reported to have shown behavioral signs of estrous during the month previous to the beginning of the experiment. During the whole study, cows were maintained in a shaded pen and were fed alfalfa hay, with *ad libitum* minerals and fresh water.

Hormonal treatment and detection of estrous

The estrous cycles of all cows were synchronized with two IM injections of 25 mg of the prostaglandin F2α (PGF2α) analogue dinoprost tromethamine (5 mL Lutalyse®; Pharmacia & Upjohn; Kalamazoo, MI) administered 11 d apart. Upon the second injection, all cows were observed continuously (24 h a day) over five consecutive days by one of four trained technicians to determine the beginning of estrous, which was considered when one cow stood passively to be mounted by another cow during ≥ 3 s for the first time. Seventeen days after the onset of the synchronized estrous period the same cows were observed continuously over seven consecutive days or until all cows had shown spontaneous estrous behavior to determine its onset as described before. Onset of synchronized and spontaneous estrous was considered to have occurred during daytime in cows that showed it between 0600 and 1800 h; conversely, onset of synchronized and spontaneous estrous during nighttime was considered to have occurred in cows that showed it between 1800 and 0600 h. The end of synchronized and spontaneous estrous was determined to have occurred by the last observation in which a cow stood to be mounted, calculating estrous length accordingly. In the present study, only cows that showed synchronized and the subsequent natural estrous were used.

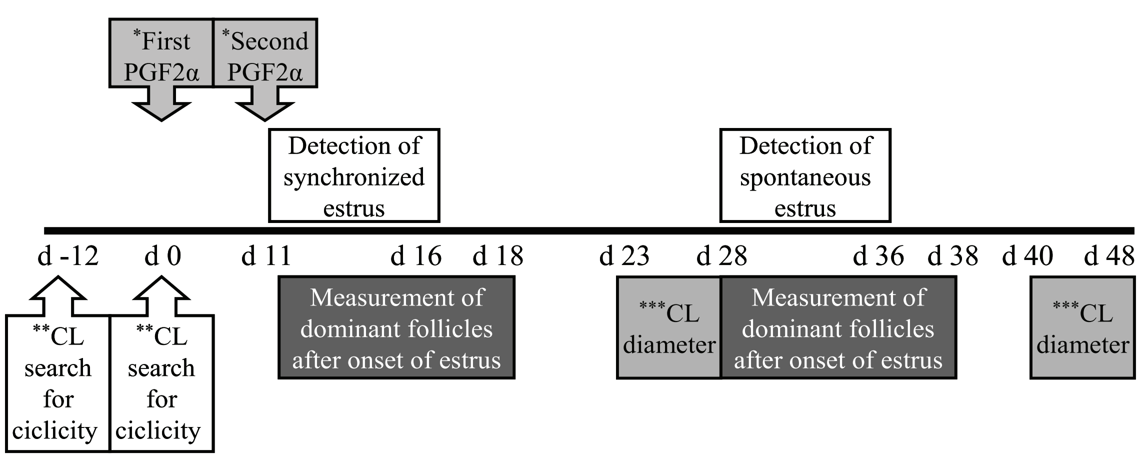

Figure 1. Timeline of measurement of ovulatory follicles and *corpora lutea* (CL) after synchronized and spontaneous estrous in Creole cows (*injection of 25 mg dinoprost tromethamine; ** ovarian cyclicity was determined when a corpus luteum was detected by ultrasonography on d -12 or 0; *** Diameter of corpus luteum was measured in each cow on day 10 after ovulation).

Measurement of ovarian dynamics

After the onset of synchronized and natural estrous both ovaries of each cow were monitored by transrectal ultrasonography by the same trained operator using a Sonovet 600 device with a 7.5 MHz linear transducer (Medison, Inc., Cypress, CA, USA). In order to identify dominant follicles (DF) and ovulation, ultrasound monitoring was carried out every 8 h until ovulation occurred. A DF was identified as the largest follicle present that increased its diameter after deviation, which was considered to happen during the middle between two ultrasound observations in which smaller (subordinate) follicles showed a decrease in diameter due to atresia (Lynch *et al.,* 2010; Sartori and Barros, 2011). Growth rate of a DF was calculated as the increase in its diameter in relation to the diameter detected in the ultrasound of the same time in the previous day (mm/d) during the deviation-ovulation interval. Ovulation was determined when the previously detected DF disappeared from one ultrasonographic observation to another. Time of ovulation was determined to have occurred in the mean time between the last observation of a DF and its disappearance (Lucy *et al.,* 1991; Sartori *et al.,* 2001), calculating the interval from onset of estrous to ovulation accordingly.

At d 10 after the detection of synchronized and spontaneous ovulation of each cow the corresponding CL was identified and measured by ultrasound. At this time of estrous cycle, a mature post-ovulation CL was considered as such when a clearly demarcated echogenic structure appeared on the ovary stroma in the same place of previous ovulation. Diameter (D) of each CL image was measured at the transversal axis of the circle-shaped structure (Hanzen *et al.,* 2000).

Statistical analyses

The proportion of cows that showed onset of synchronized and natural estrous during day (0600 to 1800 h) or night (1800 to 0600 h) were compared using the LOGISTIC procedure of SAS (SAS Inst. Inc, Cary, NC, USA). Data of estrous length, diameter of DF at deviation, maximum diameter reached by a preovulatory DF, DF growth rate, time from onset of estrous to ovulation, and diameter of CL were analysed by ANOVA under a completely randomized design using proc GLM of SAS program (SAS Inst. Inc, USA) in which the origin of estrous (induced by PGF2α or natural) was used as treatment, and each cow was considered as an experimental unit. Normality of distribution of data was verified by Levene's test. Results are presented as mean values ± SD. Comparisons of means were performed with Tukey tests and differences in mean value comparisons were considered as statistically significant at the $p \leq 0.05$ level whereas a trend was accepted to occur if $0.05 < p < 0.10$.

Results

Twenty-two of 30 cows (73.3%) showed synchronized estrous behavior after the second PGF2α injection; the same cows showed a subsequent spontaneous estrous 21.1 (± 1.2; range 19 to 23) d later. Occurrence of synchronized and naturally-occurring estrous during the day and night is shown in Table 1. The proportion of cows that showed heat during daytime was similar in both the synchronized and spontaneous estrous groups ($p > 0.1$). Also, the proportions of cows with synchronized and spontaneous estrous showing heat during the night were similar ($p > 0.1$). The overall proportion of cows that showed onset of estrous, regardless of its origin, was higher during nighttime (31 vs. 13 cows; $p < 0.01$).

Table 1. Percentage of Creole cows with onset of synchronized and spontaneous estrous during day (0600 to 1800 h) and night (1800 to 0600 h).

		Time of onset of estrous	
	N	0600 to 1800 h	1800 to 0600 h
Synchronized estrous*	22	8 (36.3 %)[a]	14 (63.7 %)[b]
Spontaneous estrous	22	5 (22.7 %)[a]	17 (77.3 %)[b]
Overall estrous	44	13 (29.5 %)[a]	31 (70.5 %)[b]

[a,b]Values within each row or column with different letters indicate significant differences ($p < 0.01$).

*Synchronized estrous after two PGF2α injections, 11 d apart.

Detailed data related to estrous duration, time from estrous to ovulation, diameter of DF at deviation, growth and maximum diameter of the preovulatory follicle, as well as CL size are shown in

Table 2. Estrous behavior after synchronization was similar to the mean duration of spontaneous estrous ($p>0.1$). Diameters of preovulatory DF at deviation during synchronized and spontaneous estrous were similar ($p>0.1$). The maximum diameter reached by preovulatory DF during synchronized estrous was similar to the diameter reached by preovulatory DF during spontaneous estrous ($p>0.1$).

The growth rate of preovulatory DF from deviation to ovulation was higher after synchronized estrous than after spontaneous estrous (0.9 ± 0.2 and 0.8 ± 0.2 mm/d, respectively; $p<0.05$). The time elapsed from the onset of synchronized estrous to ovulation was longer than the time elapsed from spontaneous estrous to ovulation (46.2 ± 8.2 and 37.6 ± 6.0 h, respectively; $p<0.01$). The mean diameter of CL at d 10 after ovulation was similar after synchronized and spontaneous estrous (13.2 ± 1.5 and 13.5 ± 1.7 mm, respectively; $p>0.1$).

Table 2. Estrous duration, time from estrous to ovulation, follicular diameter at deviation, growth rate and maximum diameter of the preovulatory follicle and *corpora lutea* (CL) of Creole cows under synchronized and spontaneous estrouses.

	Origin of estrous						
	Synchronized			Spontaneous			
	Mean	**SD**	**Range**	**Mean**	**SD**	**Range**	**p value**
Estrous duration (h)	15.3	5.5	6 to 26	14.1	2.8	8 to 20	0.3982
Diameter of preovulatory follicle at deviation (mm)	5.1	0.5	4 to 6	4.7	0.7	4 to 6	0.0587
Maximum diameter of the preovulatory follicle (mm)	9.9	1.2	8 to 13	10	0.9	8 to 12	0.5961
Growth rate of the preovulatory follicle (mm/d)*	0.99	0.2	0.6 to 1.5	0.81	0.2	0.4 to 1.2	0.0112
Time from onset of estrous to ovulation (h)	46.2	8.2	28 to 60	37.6	6.0	28 to 48	0.0003
Diameter of corpus luteum (mm)**	13.2	1.5	11 to 16	13.5	1.7	11 to 16	0.4771

*Growth rate from deviation to ovulation.

** Diameter of corpus luteum was measured on d 10 after ovulation.

SD (standard deviation).

Discussion

The mainstay of estrous synchronization with PGF2α is its ability to induce regression of mature luteal tissue, which must be at least 5 d old at the time of injection (Wiltbank *et al.,* 1995), although it has been reported that between days 5 and 9 of the estrous cycle PGF2α may cause partial luteal regression with subsequent recovery of the CL function (Pinheiro *et al.,* 1998). In the present study all cows had active ovaries and were treated with the PGF2α analogue twice at an interval of 11d, therefore, we assumed that they would have had a CL that was susceptible to the PGF2α. The percentage of Creole cows that showed estrous after synchronization with PGF2α agrees with previous studies that report 70 to 90% of *Bos taurus* animals (Odde, 1990; Tanabe and Hann, 1983) and more than 70% of *Bos indicus* cattle in estrous after similar hormonal treatments, although some authors report that estrous occurrence after PGF2α rarely surpasses 40% in *Bos indicus* cattle (Landivar *et al.,* 1985).

The present study does not show an effect of estrous origin (induced or spontaneous) on estrous onset during daytime. These results agree with those by Pinheiro *et al.* (1998), which reported that Nelore cattle showed heat during day or night, regardless of the type of estrous. In this regard, it is important to notice that luteal regression may differ after exogenous PGF2α injection, when compared to the effect of natural PGF2α, given the fact that a larger dose or a second dose may be required in order to achieve complete luteolysis, with a subsequent occurrence of estrous behaviour at any time of day. Standing heat can occur any time in a 24-hour period. However, the most likely time for a cow or heifer to show heat signs is at night, which coincides with the results obtained in the present study. Furthermore, it has been reported that season can have an influence, with more cows showing heat at night in hot weather and more showing heat during the day in cold weather (Britt *et al.,* 1986). The climatic conditions during the present study were not extreme in a hot or cold way, thus, it does not seem feasible that temperature affected the time of estrous onset. Housing conditions can also have an effect on the distribution of heat during a 24-hour period, as crowded conditions and high stress environments may reduce mounting activity during daytime (Pinheiro *et al.,* 1998). In this regard, it should be noted that the Creole cows used in the present study are not usually subjected to confinement and handling techniques, thus, such stress-causing factors may have influenced the onset of behavioral estrous, as reported by other authors (Cooke *et al.,* 2009; 2012), with overall higher proportion of cows in heat during the night. The higher incidence of Creole cows showing standing heat during the night may make its detection difficult, thereby limiting the use of artificial insemination and other reproduction techniques.

Although there was no difference in the present study between diameter of DF at deviation or prior to ovulation after synchronized or spontaneous estrous, the mean overall maximum diameter of follicles were considerably smaller than those of other *Bos taurus* (up to 13.6, 17.2 and 15.8 mm; Sartori *et al.,* 2001; Townson *et al.,* 2002; Bleach *et al.*, 2004; respectively) and *Bos indicus* breeds (up to 12.8 mm; Sartori and Barros, 2011). This indicates that ovulatory follicles in Creole cows may be capable of achieving functional dominance and ovulatory capacity at a smaller diameter than what has been reported in other commercial bovine breeds. Additionally, in our study, the similar duration of both naturally-occurring and hormonally-induced estrous may indicate that DF of Creole cows with similar size at deviation and prior to ovulation in both types of estrous behave similarly in estradiol synthesis and secretion, causing similar estrous behavior in terms of duration, as noted by At-Taras and Spahr (2001). Interestingly, the longer overall estrous duration observed in the present study differ from data reported by other researchers, who found numerically shorter periods of total mounting activity in Holstein females (5.83, 7.2, and 9.5 h; At-Taras and Spahr, 2001; Piggot *et al.,* 1996; and Walker *et al.,* 1996; respectively). Although a numerically longer time of standing estrous behaviour seem to be a particularity of Creole cattle, the estrous display variations in the different experiments could be attributed to different trial conditions (e.g. space availability for mounting, type of flooring, and estrous detection techniques, among others) and therefore needs further experimental evaluation, as other researchers report estrous duration ranging from 12 to 16 h after luteolysis in other breeds (Stevenson *et al.,* 1998).

The overall growth rate of the dominant preovulatory follicles was lower when compared with those observed by other researchers in European breeds (up to 1.7 and 2.2 mm/d; Fortune *et al.,* 1988; and Murphy *et al.*, 1990, respectively). The higher follicular growth rate after synchronized estrous in the present study could be attributed to variations in gonadotropin levels that can exist among bovine breeds (Parker *et al.,* 2003; Burns *et al.,* 2005). Additionally, the increase in DF growth rate after PGF2α could indicate loss of negative feedback from progesterone luteal hormone (LH; Atkins *et al.,* 2010). Therefore, an increase in LH pulse frequency could drive follicle growth and estradiol production (Fortune, 1994). The different growth rates after synchronized and spontaneous estrous could have important implications for artificial insemination (AI) timing following synchronization treatments, as timing may affect oocyte capacity to be fertilized (Atkins *et al.,* 2010). This suggests that the best AI timing may differ in Creole cows compared to other breeds.

In the present study, the overall time elapsed from estrous onset to ovulation was higher to those reported in other studies in dairy and beef cattle, for which time from estrous onset to ovulation was up to 32.9 h (Wiltbank *et al.,* 1967) or averaged 20-30 h (Roelofs *et al.,* 2006; Peter *et al.,* 2009), while in averaged 24.4 h in other *Bos* species (*Bos frontalis*; Mondal *et al.,* 2006) Although synchronization in this study did have an effect on the subsequent ovulation timing, similar ovulation times have been reported between

PGF2α-induced and spontaneous estrous (Cárdenas *et al.,* 1991; Pinheiro *et al.,* 1998). The longer time from onset of synchronized to ovulation, when compared to the spontaneous estrous may be due to incomplete luteolysis after PGF2α or interference of the hormone protocol with follicular dynamics (Drillich *et al.,* 2000). Additionally, it should be noted that longer intervals between prostaglandin injections than the one used in the present experiment (11 d) may lead to increased luteal regression response with higher estrous concentration and possibly shorter time to ovulation, as reported by Folman *et al.,* (1990).

The origin of the estrous (synchronized or spontaneous) did not affect the maximum size reached by the subsequent CL 10 d after ovulation. This seems logical, given the facts that the maximum diameter of the preovulatory follicle was similar in both estrous types and there is positive relation between the ovulatory follicle size and the maximum size of CL (Jaiswal *et al.,* 2009). A possible explanation for this is that with a greater follicular size, serum concentrations of estrogen and LH, as well as LH receptors in theca and granulosa cells increase (Peter *et al.,* 2009), as it is well known that LH plays an important role in the normal development and function of luteal cells (Nisweinder *et al.,* 2000). In cattle, release of LH pulses before its preovulatory surge is required for follicle maturation, which in turn allows an adequate CL development (Quintal-Franco *et al.,* 1999). It is generally assumed that there is a relationship between CL volume and progesterone serum concentrations (Grygar *et al.,* 1997; Peter *et al.,* 2009; Lüttgenau *et al.,* 2011). Considering that *corpora lutea* after both synchronized and spontaneous estrous in the present study were similar in size, it is assumed that progesterone secretion might also be similar during the luteal phase in diestrous.

In conclusion, most Creole cows show heat during the night, regardless of estrous origin. Additionally, the growth rate of preovulatory follicles is higher after PGF2α synchronization and the time from onset to ovulation is longer in synchronized estrous. Although further research is required, these findings could indicate that timing of artificial insemination after estrous onset in Creole females must be reevaluated, especially after PGF2α synchronization.

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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