

Open Cows in a Beef Cattle Enterprise Managed in a Seasonal Breeding Program? An Appraisal of Their Reproductive Performance Based on Body Fat Reserves

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Abstract

A comparison between two distinct levels of back fat thickness in open cows, females not becoming pregnant during the previous breeding season, was carried out in order to evaluate their reproductive performance prior to the next natural mating in a seasonal breeding program. Thirty open cows from the previous breeding season were divided evenly by age and back fat thickness (BFT) in two groups. HBFT (high back fat thickness ≥ 70 mm) and LBFT (low back fat thickness ≤ 70 mm), values statistically different between groups ($P < 0.05$). The study was divided into three phases in which the formation of CL and follicular dynamics were recorded by ultrasound and progesterone. Also, serial monitoring of glucose, urea and triglycerides and back fat thickness were studied. A fertile bull previously approved from a breeding soundness evaluation was introduced after the third phase and remained with the herd for 90 days. No differences were found in the follicular dynamics; the number of animals cycling in the two groups and fertility were also similar (80%). Differences in urea and triglycerides were found in cows with high scores of BFT. In conclusion, the decision of keeping open cows to the next breeding season must be based on an economical advantage to the farmer.

Keywords

Back Fat Thickness, Corpus Luteum, Follicular Dynamics, Tropics, Zebu Cattle

1. Introduction

There are innumerable causes for the cows not becoming pregnant during the breeding season and therefore these cows should wait for the next breeding season. Commonly in practice are those cows denominated open cows. Among them, are the poor nutritional status of the dam following calving [1], the interference of the calf due to intense suckling [2] or poor fertility of the bull [3]. Ibendahl *et al.* [4] using a mathematical model, concluded that the only justification to keep animals not pregnant until the next breeding, would be if the feeding cost would be low, the value of replacement animals is high, or the selling of the open cows or their offspring was also low. In a recent study, Martínez *et al.* [5] compared the fertility of cows recently calved compared to open females from the prior breeding season, concluding that, although overall fertility was about 80% in both groups, postpartum cows became pregnant quicker than the barren ones, suggesting that it is economically questionable to keep the latter.

Monitoring the metabolic status of the animals can be cumbersome, one of the most published methodologies is the measurement of metabolites such as glucose, triglycerides, non-esterified fatty acids, and urea [6] [7] [8]. Alternatively, one can use the body condition score, and technique, which manually or visually, can predict the health status of the animal. However, when compared to the measurement of dorsal body fat using ultrasound, the former has proved to be not very accurate [9] [10]. Martínez *et al.* [5] in fact, postulated that the monitoring of back fat thickness could be used as a good predictor of reproductive performance in the breeding season.

The objective of the present study is to compare open cows with two distinct amounts of back fat thickness prior to natural mating to evaluate its effect on reproductive performance.

2. Material and Methods

2.1. Location

The present study began 30 days prior to the start of the annual breeding season in the F1 Heifer Production Module of the Centre for Teaching, Research and Extension in Tropical Animal Husbandry belonging to the Faculty of Veterinary Medicine of the National Autonomous University of Mexico, which is located in Tlapacoyan, Veracruz, Mexico at 20°4'N and 97°3'W (Figure 1). The climate is warm-humid with no definite dry season. The average annual rainfall is 1840 mm³ and the average temperature varies in a range of 14°C to 35°C.

2.2. Animals

Thirty multiparous Brahman cows clinically healthy with ages ranging from 4 to 8 years old which remained open from the previous seasonal breeding program were used. All animals were kept under a rotational grazing system in pastures composed of an association of *Cynodon niemfuensis* (star grass), *Paspalum* spp.



Figure 1. (A): Geographical location of the study site. (B): Experimental location of the study site.

and *Axonopus* spp. No supplement was procured to the animals and water and salt were provided ad libitum.

2.3. Experimental Design

The thirty open cows were divided evenly by age and back fat thickness (BFT) in two groups, group HBFT (high back fat thickness ≥ 70 mm) and LBFT (low back fat thickness ≤ 70 mm), being BFT values statistically different ($P < 0.05$) on day -3 and -1 prior the trial using an ultrasonic device (Aloka SSD 500, Tokyo, Japan) with a 3.5 MHz frequency convex transducer.

The study was divided in three different follicular wave scenarios (**Figure 2**): 1) In the first, an IM injection of 25 mg of Lutalyse® (Zoetis, Mexico) was applied (day 0) in cows with corpus luteum present; in those without corpus luteum, an IM placebo was applied. Normal follicular surges were monitored by transrectal ultrasonographic examination of the ovaries on days (0, 2, 4, 6, 9, 16, 18, 20, 25, 27, 29 and 31) recording dominant follicle dynamics and the presence of a corpus luteum using an ultrasonic device (Aloka SSD 500, Tokyo, Japan) with a 7.5 MHz. Furthermore, to determine that animals in phase one did have a CL present or absent, ultrasounds were performed until the formation of new corpora lutea in animals receiving a dose of prostaglandin as opposed to those in possible anestrus. These observations were recorded whether they formed corpus luteum or continued without the presence of a CL (day 9). 2) In the second, a 1.9 gm of CIDR® (Zoetis, Mexico) was introduced to regulate the cyclicity in all

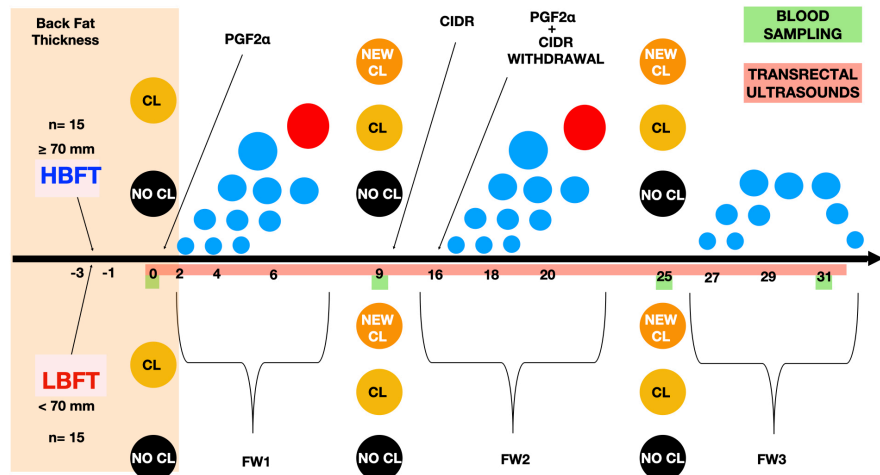


Figure 2. Scheme of the experimental activities. FW = Follicular wave, CL = corpus luteum present, NO CL = absence of corpus luteum and New CL: following the use of prostaglandin.

individuals with or without a corpus luteum present (day 9). On day 16 the device was removed and 25 mg of Lutalyse® (Zoetis, Mexico) IM was injected in all animals and the follicular dynamics and the presentation of estrus on days 18, 19 and 20 until the formation of new corpora lutea (day 25) was recorded. 3) The third follicular wave were evaluated (days 25, 27, 29 and 31) regardless of the animals with a CL present. A fertile bull previously approved from a breeding soundness evaluation was introduced after the third phase and remained with the herd for 90 days.

2.4. Ovarian Activity

The Ultrasonography

Transrectal ultrasonographic examination of the ovaries was performed on days (0, 2, 4, 6, 9, 16, 18, 20, 25, 27, 29 and 31) recording dominant follicle dynamics (**Figure 3**) and the presence of a corpus luteum (**Figure 4**) using an ultrasonic device (Aloka SSD 500, Tokyo, Japan) with a 7.5 MHz frequency linear transducer [11].

Serum progesterone

Blood samples were obtained by coccygeal vein puncture on experimental days (0, 9, 25 and 31), centrifuged at 3500 rpm and serum was stored in vials at -20°C . Serum plasma progesterone (P4) levels were recorded, with ELISA kits (DRG® Progesterone ELISA, Germany) as an indicator of the presence of corpus luteum with 3.3% intra-assay CV. P4 levels higher than 1 ng/mL in serum [12].

2.5. Nutritional Status

Back fat thickness

Back fat thickness was measured in the rump area on experimental days (−3 and −1) in the gluteal region at a midpoint between the coxal and the ischial



Figure 3. Ultrasound image of a bovine ovary with dominant and subordinate follicles.

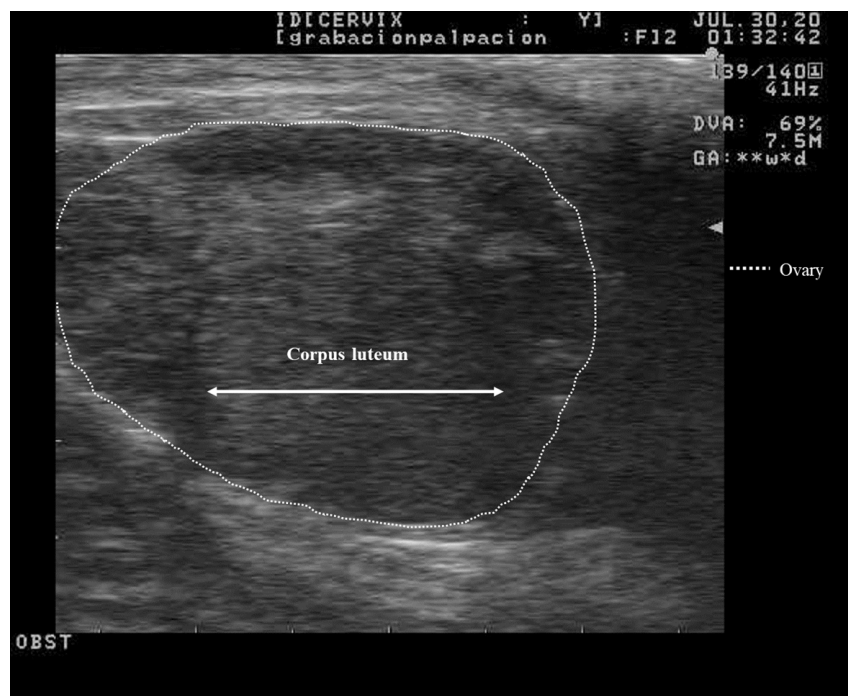


Figure 4. Ultrasound image of a bovine ovary with corpus luteum.

tuberosities, 2 - 3 cm above the trochanteric region [13], using an ultrasonic device (Aloka SSD 500, Tokyo, Japan) with a 3.5 MHz frequency convex transducer.

Blood metabolites

Blood samples were collected on experimental days (9, 25 and 31) by puncture of the coccygeal vein, centrifuged at 3500 rpm and the serum stored in vials at

–20°C. Glucose, urea and triglycerides were measured in duplicate using commercial kits (BioSystem®; Barcelona-Spain) with 4.4%, 2.1% and 5% intra-assay CV, respectively [8].

2.6. Pregnancy Diagnosis

Pregnancy diagnosis was performed every two weeks starting one month after the introduction of the bull to record as close as possible the actual time of gestation. It was carried out by transrectal ultrasonography, using an ultrasound (Aloka SSD 500, Tokyo, Japan) with a linear 7.5 MHz transducer. The pregnancy was confirmed by the presence of an amniotic vesicle, the embryo itself, and/or its heartbeat [14].

2.7. Statistical Analysis

Variations in the values recorded for ovarian structures and metabolites was evaluated by ANOVA test for repeated measures according to group and follicular wave. A non-parametric survival analysis by Kaplan-Meier curves was undertaken to compare cows becoming pregnant early between the breeding programs and a log-rank test was used to determine their significance. Differences in pregnancy percentage between the two groups were analyzed with 2×2 contingency tables using Fisher's test [15]. All statistical tests were done with SAS 9.4 considering $P < 0.05$ statistically different

3. Results

Follicular Wave 1

During this stage the size of the dominant follicles was not different ($P = 0.9560$) between the HBFT and LBFT groups with 10.36 ± 2.915 mm and 10.69 ± 2.046 mm, respectively (Figure 5). Corpus luteum size was not different between groups ($P = 0.2850$), registering 14.00 ± 3.609 mm for the HBFT group and 11.89 ± 1.533 mm for the LBFT group (Figure 6). P4 levels were 7.225 ± 9.906 ng/mL for the HBFT group and 3.878 ± 4.832 ng/mL for the LBFT group, there

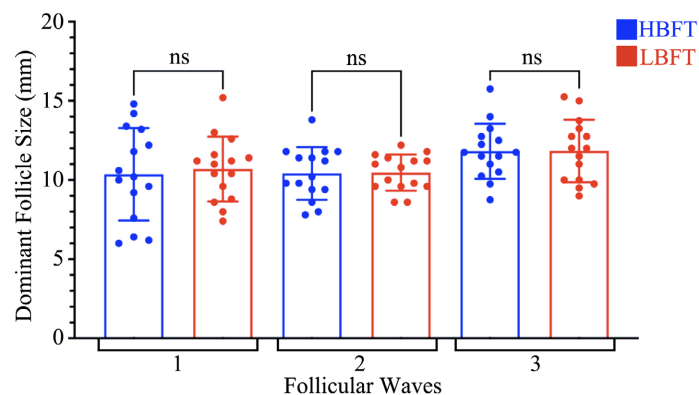


Figure 5. Comparison of the dominant follicular size in three follicular waves between HBFT (Blue) and LBFT (Red) groups. Dots are the mean values for each case, top of the bar denotes the mean and the lines the standard deviation.

was no difference between groups ($P = 0.6370$). The number of cows with corpus luteum present at the end of the first follicular wave was 13/15 for the HBFT group and 9/15 for the LBFT group, there was no difference between groups ($P = 0.2148$). Glucose levels were 72.27 ± 11.61 mg/dL for the HBFT group and 72.20 ± 8.693 mg/dL for the LBFT group, there was no difference between groups ($P > 0.9999$). Triglyceride levels presented a statistical difference between groups ($P < 0.0001$) with 209.60 ± 39.23 mg/dL in the HBFT group and 162.1 ± 42.52 mg/dL in the LBFT group. Urea levels were 52.83 ± 7.493 mg/dL for the HBFT group and 31.07 ± 5.989 mg/dL for the LBFT group, showing a statistical difference ($P < 0.0001$) (Table 1).

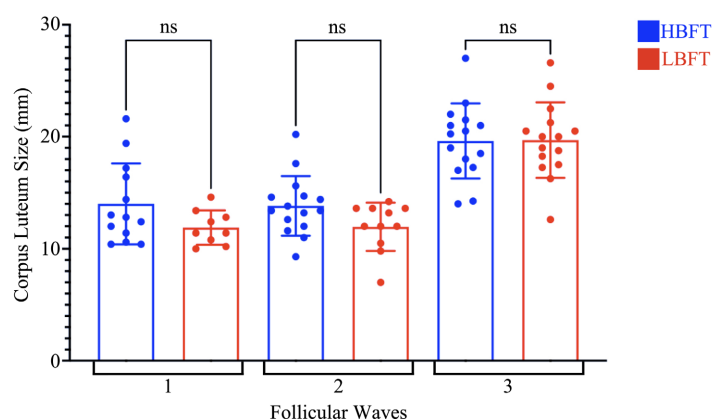


Figure 6. Comparison of corpus luteum size in three follicular waves between HBFT (Blue) and LBFT (Red) groups. Dots are the mean values for each case, top of the bar denotes the mean and the lines the standard deviation.

Table 1. Comparison of glucose, triglycerides, and urea between HBFT (≥ 70 mm BFT) and LBFT (< 70 mm BFT) in open cows at three different follicular waves prior to a breeding season.

	HBFT		LBFT		P
	Mean	SD	Mean	SD	
1st Follicular Wave					
Glucose (mg/dL)	72.27	11.61	72.20	8.693	>0.9999
Triglycerides (mg/dL)	209.6	39.23	162.1	42.52	<0.0001
Urea (mg/dL)	52.83	7.493	31.07	5.98	<0.0001
2nd Follicular Wave					
Glucose (mg/dL)	78.30	20.96	73.20	7.32	0.2783
Triglycerides (mg/dL)	207.6	38.17	179.4	32.81	0.0104
Urea (mg/dL)	49.87	7.688	28.81	7.92	<0.0001
3rd Follicular Wave					
Glucose (mg/dL)	69.11	10.77	69.42	7.279	0.9995
Triglycerides (mg/dL)	210.90	37.15	173.7	29.98	0.0004
Urea (mg/dL)	50.96	3.840	34.25	7.661	0.0007

Follicular Wave 2

During this stage the size of the dominant follicles was not different ($P = 0.9998$) between the HBFT and LBFT groups with 10.41 ± 1.660 mm and 10.47 ± 1.143 mm, respectively (**Figure 5**). Corpus luteum size was not different between groups ($P = 0.2850$), registering 13.830 ± 2.656 mm for the HBFT group and 11.95 ± 2.145 mm for the LBFT group (**Figure 6**). P4 levels were 4.905 ± 7.023 ng/mL for the HBFT group and 2.217 ± 2.975 ng/mL for the LBFT group, there was no difference between groups ($P = 0.7749$). The number of cows with corpus luteum present at the end of the first follicular wave was 15/15 for the HBFT group and 11/15 for the LBFT group, there was no difference between groups ($P = 0.0996$). Glucose levels were 78.30 ± 20.96 mg/dL for the HBFT group and 73.20 ± 7.328 mg/dL for the LBFT group, there was no difference between groups ($P = 0.2783$). Triglyceride levels presented a statistical difference between groups ($P = 0.0104$) with 207.6 ± 38.17 mg/dL in the HBFT group and 179.4 ± 32.81 mg/dL in the LBFT group. Urea levels were 49.87 ± 7.688 mg/dL for the HBFT group and 28.810 ± 7.928 mg/dL for the LBFT group, showing a statistical difference ($P < 0.0001$) (**Table 1**).

Follicular Wave 3

During this stage the size of the dominant follicles was not different ($P > 0.9999$) between the HBFT and LBFT groups with 11.820 ± 1.744 mm and 11.830 ± 1.972 mm, respectively (**Figure 5**). The size of the corpus luteum showed no statistical difference between the groups ($P = 0.9413$), registering 19.620 ± 3.351 mm for the HBFT group and 19.700 ± 3.370 mm for the LBFT group (**Figure 6**). P4 levels were 10.38 ± 12.56 ng/mL for the HBFT group and 8.055 ± 10.060 ng/mL for the LBFT group, there was no difference between groups ($P = 0.8412$). The number of cows with corpus luteum present at the end of the first follicular wave was 15/15 for the HBFT group and 15/15 for the LBFT group, there was no difference between groups ($P = 1$). Glucose levels were 69.11 ± 10.77 mg/dL for the HBFT group and 69.42 ± 7.279 mg/dL for the LBFT group, there was no difference between groups ($P = 0.9995$). Triglyceride levels presented a statistical difference between groups ($P = 0.0004$) with 210.9 ± 37.15 mg/dL in the HBFT group and 173.7 ± 29.98 mg/dL in the LBFT group. Urea levels were 50.96 ± 3.840 mg/dL for the HBFT group and 34.25 ± 7.661 mg/dL for the LBFT group, showing a statistical difference ($P = 0.0007$) (**Table 1**).

Overall pregnancy across the breeding season was 86% for HBFT group (12/15) and 86% for the LBFT group (12/15) (**Figure 7**), existing no differences between both groups during the Breeding Season ($P = 0.7256$).

4. Discussion

Unexpectedly, there were no differences in the size of the dominant follicle throughout the three stages of the study. Several essays [16] [17] have shown that in order to have differences in the size of the dominant follicle, it is almost mandatory that the body condition score of the animals would be affected by diet

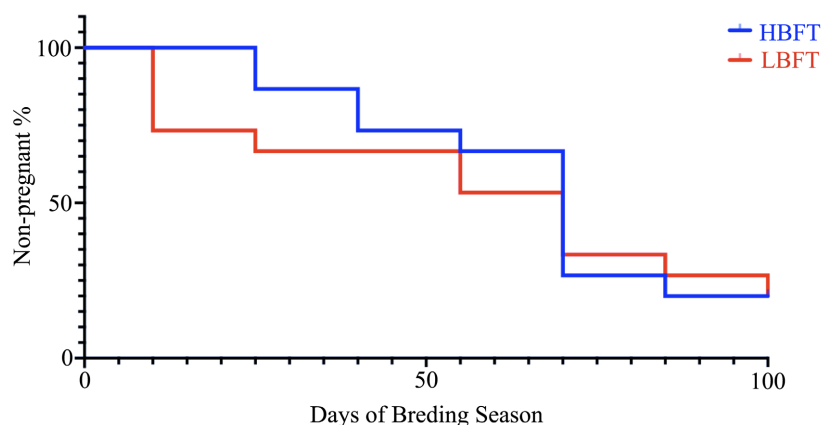


Figure 7. Comparison between the survival curves of HBFT (Blue) and LBFT (Red) groups for being non-pregnant at different times of the breeding season.

[17] or intensive suckling [11] [18]. Unfortunately, it was not feasible to measure all the follicles present in the ovary, nor the periodicity of the observations [11]. Nonetheless, judging by the number of cows already cycling at the start of the experiment, one could assume that the follicular waves in the ovaries of the experimental animals was reasonably regular [19]. Similarly, the number of cows with a corpus luteum at the onset of the observations was alike in the two groups. Our hypothesis thus, had to be rejected, as cows not pregnant during a seasonal breeding season and remaining open until the next mating program, were not erratic in their cyclicity even in those with less back fat thickness [20]. This in turn, would jeopardize their reproductive performance. This was not the case, which could not be explained by the age or metabolic profile of the animals. Only cows with a robust back fat thickness, were different in the concentration of triglycerides and urea during the three phases of the study which is expected in heavier cows [21].

A logical explanation of the similarities in the pregnancy rates either by accumulative percentages of overall pregnancy, could be, that barren cows, did not have the presence of a calf at foot nor the time required to recuperate from calving [22] [23]. Suckling at will, has been shown to be one of the most important deterrents to the onset of ovarian activity after calving [17] [24]. In effect, Mondragón *et al.* [11] have shown that the size of the biggest follicle recorded can be affected by the management of the cows, in fact, a regime of restricted suckling favours the earlier growth of follicles and the prompt restoration of ovarian activity [25]. Equally, Sa Filho *et al.* [18] have reported that fertility is closely related to the size of the follicle following synchronization.

Keeping open cows until the next seasonal breeding program, has been questioned recently using an economic criterion to judge the advantage of keeping barren cows until the next breeding season. In a recent study, Martínez *et al.* [5] compared the fertility of cows recently calved compared to open females from the prior breeding season, concluding that, although overall fertility was about 80% in both groups, postpartum cows became pregnant quicker than the barren

ones, suggesting that it is economically questionable to maintain the latter. Our results, support previous data that keeping open cows in a seasonal breeding program is economically rather debatable, the present assay illustrates that increasing the number of barren cows to be maintained until the next breeding season is rather questionable [4].

5. Conclusion and Recommendations

In conclusion, from the reproductive viewpoint, there are no differences in fertility in open cows either with different backfat thicknesses or in their metabolic profile. The decision of keeping open cows to the next breeding season must be based on an economical advantage to the farmer.

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Ethics Approval

All the animals and the procedures were reviewed and approved by the Ethical Committee for Experimentation in Animals of the Faculty of Veterinary Medicine and Zootechnics of the National Autonomous University of Mexico, in accordance to The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Author Contributions

Martinez Jose Francisco: performed the experimental study, analysed data, and manuscript writing. Ortiz Pablo: performed the experimental study and data collection. Galina Carlos Salvador: experimental design secure funding writing and editing. Corro Manuel and Rubio Ivette: performed the experimental study in the field. Pérez-Torres Libia: secure funds for publishing the scientific paper, data analysis, review and writing. All authors contributed to the article and approved the submitted version.

Data Availability Statement

The raw data supporting the results and conclusions of this article will be made available by the authors, without reservation

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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