



EFFECT OF BODY CONDITION SCORE ON ESTRUS AND OVARIAN FUNCTION CHARACTERISTICS OF SYNCHRONIZED BEEF-MASTER COWS

[EFECTO DE LA EVALUACIÓN DE LA CONDICIÓN CORPORAL SOBRE EL ESTRO Y CARACTERÍSTICAS DE LA FUNCIÓN OVÁRICA EN VACAS DE CARNE SINCRONIZADAS]

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SUMMARY

The effect of body condition score (BCS) on estrus characteristics and ovarian function was evaluated in 44 synchronized Beef-Master cows. Weaned adult cows in anestrus were grouped according to their BCS as BCS1-2 (n = 13), BCS3-4 (n = 17) and BCS5-6 (n = 14). All cows were synchronized using norgestomet implants plus estradiol valerate for 9 days. The estrus detection began 24 hours after implant removal and it was detected every 6 hours for 72 hours. The time from implant removal to the onset of estrus (TIR) and the duration of estrus were registered. All animals were examined 4 times with a transrectal ultrasound kit to determine the size of the follicles (6 to 9 mm or > 9 mm), the diameter of the largest follicle (DLF), ovulation rate, ovulation rate relative to estrus detection (ORE) and ovulation rate relative to the follicle size (ORF). The effect of BCS on TIR, the duration of the estrus, the number of follicles of size 6 to 9 mm or > 9 mm, and DLF was determined using analysis of variance. The effect of BCS on ovulation rate, ORE and ORF, were determined using Chi-square tests. There were no differences ($P > 0.05$) between TIR, duration of estrus and follicle sizes between BCS groups. The DLF increased from 9.8 to 12.9 mm, the ovulation rate from 7.7 to 85.7%, the ORE from 0 to 100% and the ORF from 16.7 to 92.9%. In conclusion, under the range of BCS of the synchronized cows, evaluated in this study, the BCS of Beef Master cows did not affect the estrus characteristics; which agree with results observed in other breeds of cattle in the tropics. However, the BCS affect follicle development and ovulation rate.

Keywords: body condition; Beef Master; estrus; follicular development; ovulation.

RESUMEN

Se evaluó el efecto de la condición corporal (CC) sobre las características del estro y función ovárica en 44 vacas Beef Master sincronizadas. Vacas adultas destetadas y en anestro se agruparon con base en su CC como: CC1-2 (n=13), CC3-4 (n=17) y CC5-6 (n=14). Las vacas fueron sincronizadas con implantes de norgestomet más valerato de estradiol por 9 días. La detección de estro comenzó 24 horas después de retirar el implante y se detectó cada 6 horas por 72 horas. Se registró el tiempo de retiro del implante hasta el inicio del estro (TRI) y la duración del estro. Los animales se examinaron 4 veces con un equipo de ultrasonido transrectal para determinar el tamaño de los folículos (6 a 9 mm o > 9 mm), el diámetro del folículo mayor (DFM), la tasa de ovulación, la tasa de ovulación relativa a la detección del estro (TOE) y la tasa de ovulación relativa al tamaño del folículo (TOF). El efecto de CC sobre TRI, la duración del estro, el número de folículos de tamaño 6 a 9 mm o > 9 mm, y DFM se determinaron usando análisis de varianza. El efecto de CC sobre la tasa de ovulación, TOE y TOF se determinó usando pruebas de Chi-cuadrada. No se encontró diferencia ($P > 0.05$) entre TRI, la duración del estro y tamaño del folículo entre grupos de CC. El DFM aumentó de 9.8 a 12.9 mm, la tasa de ovulación de 7.7 a 85.7%, la TOE de 0 a 100% y la TOF de 16.7 a 92.9%. En conclusión, bajo el rango de CC de las vacas sincronizadas, utilizadas en este estudio, la CC de las vacas Beef Master no afectó las características estrales, lo cual concuerda con resultados observados en otras razas de ganado en los trópicos. Sin embargo, la CC afectó el desarrollo de los folículos y la tasa de ovulación.

Palabras clave: condición corporal, Beef Master, estro, desarrollo folicular, ovulación.

INTRODUCTION

Annual calf production in the tropics is low due to the late sexual maturity of heifers, prolonged postpartum anestrus (>200 days) and long calving intervals (>450 days) of cows (Nogueira, 2004; Abeygunawardena and Dematawewa, 2004). This situation is probably caused by high temperature and humidity, the presence of parasites, high variation in the quantity and quality of pasture and loss of cow body condition score during lactation (Delgado *et al.* 2004; Aban *et al.*, 2008).

In the tropics, season of calving, body condition score and suckling of calves are among the main factors that influence the length of the anestrus period. Under the extensive management conditions of Southeastern Mexico, Aban *et al.* (2008) in Zebu and crossbred cows reported 40% pregnancy rate at 120 days postpartum. Delgado *et al.* (2004) in the same breed type of cows found a pregnancy rate of 37%, also at 120 days postpartum, and 63% of cows in anestrus. In both studies, cows were lactating with a calf at foot and more than 50% of them had low body condition score. So it was likely that cows were in estrus and ovulated after weaning.

In the tropics, one strategy to decrease the length of anestrus is the use of hormones to induce and/or synchronize estrus in cows (Hattab, 2000). However, results may vary depending on the breed or body condition score (Silva-Mena *et al.*, 2002). The reduction of food consumption pattern and nutritional status of the cows in estrus synchronization programs, affect the expression of estrus, due to the lack of growth and maturation of ovarian follicles caused by a negative energy balance. Cow body condition score (BCS) reflects the nutritional status of the herd and is used as a criterion to measure the response to hormonal treatment to stimulate the resumption of ovarian activity in weaned cows when the negative effects of suckling has been suppressed.

Some studies on reproductive events surrounding the induction and/or synchronization of estrus in *Bos indicus* cattle have been developed in the tropics (Ross *et al.*, 2004; Kim *et al.*, 2005; Colazo *et al.*, 2007). However there is little information on body condition and follicle development and its relationship with ovulation rate in *Bos taurus* beef cattle under tropical conditions. The objective of this study was to evaluate the effect of BCS on the onset of estrus, follicular development and ovulation rate in synchronized Beef Master cows in Southeastern Mexico.

MATERIALS AND METHODS

The study was carried out during April to June (dry season) in a Beef-Master herd located in eastern Yucatán, Mexico. The climate is classified as AW₀, with summer rains, with a mean annual temperature of 26.3 °C, rainfall of about 469 mm and annual average relative humidity ranging from 66% in March to 89% in December (INEGI, 2005).

Forty-four adult weaned Beef-Master cows with an average of 243 ± 21 days after calving were used. Cows were managed on paddocks of Guinea grass (*Panicum maximum*) under rotational grazing from 17:00 to 7:00 hours. The remaining hours of a day the animals were housed in a “corral” for watering, minerals, salts supplementation and general management.

Ovarian inactivity of cows was measured by the absence of a corpus luteum, checked twice with a 15 days interval, using a real time ultrasound kit (Pie Medical Falco 100, Maastricht, Netherlands).

Body condition score

To assess cow BCS, a visual scheme proposed by Ayala *et al.* (1995) was used, which is based on a 9-point scale, where 1 corresponded to an emaciated and 9 to an obese cow. Thereafter, according to their BCS, cows were grouped in three categories: cows with BCS1-2 (n = 13), cows with BCS3-4 (n = 17) and cows with BCS5-6 (n = 14).

Timing and estrus detection

For estrus induction and synchronization, a subcutaneous implant of Norgestomate (Crestar, Intervet, Mexico) was used in each cow for 9 days. Cows were also injected 3 mg of Norgestomet plus 5 mg estradiol valerate intramuscularly. After 24 hours of the implant removal, estrus was visually detected every 6 hours for 72 hours (40 minutes each time). The criterion to identify a cow in estrus was the observation of the homosexual behavior of cows. The onset of estrus was determined when a cow allowed to be mounted the first time, and the end of estrus when the cow did not accept to be mounted. The duration of estrus was determined based on the mean time of two consecutive observations.

Identification of ovarian structures

An ultrasound kit performed the identification of ovarian structures, via rectal. The first diagnosis was made the day of implant removal and then every 24 hours, until one day after the end of estrus. Sheet records were used for each cow, recording the number and diameter of the follicles. Follicles were

classified in two categories: those with a diameter of 6 to 9 mm, and those with a diameter > 9 mm (Guzeloglu *et al.*, 2001). The ovulation was identified by the presence of a corpora luteum, by ultrasound, on day 11 after implant removal, and confirmed when the plasma progesterone concentration of the cow was > 1 ng / ml (Diaz *et al.*, 2002).

Plasma concentrations of progesterone were measured in blood samples taken from the coccygeal vein. Blood samples were collected in vacutainer tubes containing EDTA and then, centrifuged at 1500 x g for 15 minutes to obtain the plasma (Burke *et al.*, 2001), which was decanted in 1.5 ml Eppendorf tubes and stored at -20° C until processed. Serum progesterone was determined by radioimmunoassay solid phase, using a L125 marker (Coat-A-Count Procedure, Diagnostic Products Corporation, Los Angeles, CA, USA). The method was highly specific for progesterone with approximately 2.0% cross-reactivity with 20 α dihydroxyprogesterone, and 0.3% with 17 α -hydroxyprogesterone. The detection limit was 0.05 ng/ml and the intra-and inter-assay coefficients of variation were 7% and 8.8%, respectively.

Statistical analysis

Analyses of variance were used to evaluate the effect of BCS group on the time from implant removal to

onset of estrus, duration of estrus, the number of follicles and the diameter of the largest follicle. To determine the effect of BCS on ovulation rate, ovulation rate with respect to the cows in estrus and with respect to the cows with follicles greater than 9 mm, Chi-square tests were used. All statistical analyses were carried out using SAS (2002).

RESULTS

The time from implant removal to estrus detection, estrus duration and number of follicles of size 6-9 mm or > 9 mm was similar for the three BCS groups (Table 1). However, the diameter of the largest follicle was largest in the cows with BCS5-6. The overall estrus rate was 61.4%. As BCS improved the percentage of cows that ovulated, the ovulation rate in relation to the cows that showed estrus and those that ovulate in relation to the follicles > 9 mm improved (Table 2). Cows with BCS5-6 had the highest ovulation rate, ovulation rate relative to cows in estrus and to those with follicles >9 mm, followed by BCS3-4 group cows. However, only the differences between the BCS1-2 group cows and the other two BCS groups were significant ($P < 0.05$).

Table 1. Means (\pm SD) by body condition score for time from implant removal to onset of estrus, estrus duration, number of follicles by size and diameter of the largest follicle in Beef Master synchronized cows.

	n	Implant removal to estrus detection (hours)	Estrus duration (hours)	Follicle size		Diameter of largest follicle (mm)
				6-9 mm	>9 mm	
BCS1-2	13	36 \pm 9.5	10.2 \pm 1.4	2.8 \pm 1.6	2.7 \pm 0.8	9.8 \pm 0.9 ^a
BCS3-4	17	36 \pm 9.0	8.1 \pm 1.0	2.3 \pm 1.9	2.0 \pm 1.1	10.8 \pm 1.3 ^{ab}
BCS5-6	14	33 \pm 6.3	10.3 \pm 1.3	4.0 \pm 4.6	2.6 \pm 1.6	12.9 \pm 1.0 ^b

^{a,b} Different literals between rows, means statistical differences ($P < 0.05$).

Table 2. Number and rates by body condition score groups of ovulated cows, ovulation rate in relation to estrus and to follicle size >9 mm in synchronized cows.

	n	Ovulated cows (%)	Ovulations in relation to estrus (%)	Ovulations in relation to follicles >9 mm (%)
BCS1-2	13	1 (7.7) ^a	0/5 (0) ^a	1/6 (16.7) ^a
BCS3-4	17	11(64.7) ^b	8/10 (80) ^b	9/13 (69.2) ^b
BCS5-6	14	13(85.7) ^b	12/12 (100) ^b	13/14 (92.9) ^b

^{a,b,c} Different literals between rows, means statistical differences ($P < 0.05$).

DISCUSSION

The rate of cows in estrus (61%) after implant removal, in this study, was higher than the 21% obtained by Velázquez *et al.* (2011) in crossbred *Bos taurus* x *Bos indicus* cows. However, the values here obtained were lower than those reported in Zebu or crossbred cows in anestrus, where the response rate to estrus after progesterone implant removal was greater than 70% (Peralta-Torres *et al.*, 2010). The low response to estrus may be due to the effect of the warm months of the year (April-June) when the present experiment was carried out. It is known that the stress caused by high temperatures may affect the sexual behavior of animals (White *et al.*, 2002).

The results of this study show that the time from implant removal to the onset of estrus was similar in the three BCS groups. This agrees with Soto-Camargo *et al.* (1999), who did not find differences ($p > 0.05$) between the time of implant removal and the onset of estrus in Brahman heifers with different BCS (3.0, 3.5 and 4.0, on a scale of 1-5). Soto-Camargo *et al.* (1999) means were 42.5 ± 10.3 , 41.0 and $39.3 \pm 8.0 \pm 3.0$ hours in the three BCS groups, respectively. In this study the mean time from implant removal to the onset of estrus was 35 ± 1.7 hours, a result that is lower (43%) than the obtained by Peralta-Torres *et al.* (2010) in Zebu cattle. The fact that no differences were found among BCS groups for the time from implant removal to the onset of estrus, suggest that BCS of cows did not affect estrus response, probably because cows responded to the effect of exogenous estrogen administered with the implant; and because estrogen influenced the expression of estrus and ovulation (Lemaster *et al.*, 1999). However, the estrus in BCS 1-2 cows were anovulatory. BCS had also no effect on duration of estrus ($P > 0.05$), result that agrees with those reported by Soto-Camargo *et al.* (1999). The latter authors found no differences in Brahman heifers with different BCS (the estrus duration means were 6.5, 8.3 and 4.4 hours). However, the overall estrus duration mean (9.5 hours) was similar to that observed in *Bos indicus* cows (10.2 ± 6.8 hours) with BCS of 2.5 (Medrano, 1996). This indicates that *Bos taurus* in the tropics shows no difference in the estrus expression compared with *Bos indicus* cows, whose estrus is of short duration. The effect of BCS group on the number of follicles of size 6-9 mm or > 9 mm, agrees with the results of Burke *et al.* (1998). They found no difference ($P > 0.05$) in the number of follicles of three different sizes in cows with two BCS: low (2.3 follicles) and high (4.4 follicles) using a scale of 1 to 5 points.

In this study it was expected that the BCS5-6 group cows had the largest follicles; however, there were not differences in their diameters among the three

groups; this probably due to the postpartum time when this study was carried out (243 days on average). According to Lucy *et al.* (1991), 243 days after calving, the body condition no longer influences the number or size of the follicle; however, other factors might also be involved. The mean of the diameter of the largest follicles was higher in the BCS5-6 group (12.9 mm) as compared to the follicles for the cows with BCS1-2 (9.8 mm). These results differ from those reported by Burke *et al.* (1998), who found no difference in the diameter of the follicles of cows with low body condition (16.3 mm) and high body condition (14.2 mm). However, the diameter of the largest follicles for BCS5-6, in this study, is similar (11 mm and 12 mm) to those reported in *Bos indicus* cows (Burke *et al.*, 2001; Calvalho, 2008; Peralta-Torres *et al.*, 2010). The diameter of the largest follicles for the BCS5-6 group cows suggests that the body condition, and therefore nutrition, might have a specific effect on the growth of follicles, because the diameter of preovulatory follicles is positively correlated with the weight of *Bos indicus* cattle (Rhodes *et al.*, 1995). It has also been observed in dairy cattle that negative energy balance influences follicular growth (Bean and Butler, 1997; Wiltbank *et al.*, 2002) and the size of the ovulated follicle (Armstrong *et al.*, 2001). This situation was evident in this study, because cows with poor BCS had follicles with small diameters. Thus, a nutritional deficiency can affect the diameter of the dominant follicle and therefore ovulation. Diskin *et al.* (2003) suggest that the nutritional status of the cow affects follicular growth, maturation and ovulation.

The highest ovulation rate, ovulation rate relative to estrus and to follicles > 9 mm are similar to those reported by Sepulveda *et al.* (2001), who assessed the body condition of cows at 60 days postpartum, and found significant difference ($P < 0.05$) in ovulation; the ovulation rates were 53% and 100% in cow with BCS of 2.5 and 2.9 in a scale of 1 to 5.

The lowest ovarian activity in the group with the lowest BCS was probably due to the small follicle sizes reached by the cows of this group. Sartori *et al.* (2001) reported that follicles smaller than 10 mm in diameter, in lactating dairy cows, are unable to ovulate due to the lack of effect of the LH hormone, but the response begins to increase when the diameter of the follicles exceeds 10 mm.

It has been shown that the nutritional status of the female affects growth and follicular diameter (Rhodes *et al.*, 1995; Armstrong *et al.*, 2001; Diskin *et al.*, 2003), follicle maturation and ovulation (Diskin *et al.*, 2003). This suggests that body condition, and therefore nutrition and follicle size, had a specific effect on the proportion of cows that ovulate (Sá Filho *et al.*, 2010). It has been established that the

nutrition acts at various levels of the system that controls reproduction (Butler, 2000) and that the main pathway by which nutrition affects follicular development is through the hypothalamic-pituitary-ovarian axis (Diskin *et al.*, 2003). It is also known that the central nervous system is capable to detect subtle changes in the availability of several metabolic compounds, and as a result, the pattern of secretion of GnRH and the gonadotropins can decrease or increase depending on the change of metabolism. The administration of a diet with low protein and energy content influence negatively the LH concentration, and therefore affect the growth, persistence and ovulation of the dominant follicle (Rasby *et al.*, 1992; Rhodes *et al.*, 1995; Rhodes *et al.*, 1996). In conclusion, under the conditions of this study, there was no effect of the body condition of Beef Master cows on estrus traits, results that agree to those observed in other cattle breeds in the tropics. However, body condition affected follicle development and ovulation rate.

REFERENCES

- Abán, J., Delgado, R., Magaña, J.G., Segura J.C. 2008. Factores que afectan el porcentaje de gestación a 120 días posparto en vacas Cebú y cruzadas con europeo en el sureste de México. *Avances en Investigación Agropecuaria*. 12: 45-56.
- Abeygunawardena H., Dematawewa C.M.B. 2004. Pre-puberal and postpartum anestrus in tropical Zebu cattle. *Animal Reproduction Science* 83-83: 373-387.
- Ayala, A., Delgado, R.; Honhold, N.; Magaña, J. 1995. A visual condition scoring scheme for *Bos indicus* and crossbred cattle. In: *Dual Purpose Cattle Production Research*. Eds. S. Anderson and J. Wadsworth. IFS (International Foundation For Science) FMVZ-UADY 1995, Proceedings of an International Workshop, March 1992, Merida, Mexico, 119-128.
- Armstrong, D.G., McEvoy, T.G., Baxter, G., Robinson, J.J., Hogg C.O., Woad, K.J., Webb, R. 2001. Effect of dietary energy and protein on bovine follicular dynamics and embryo production in vitro: Associations with the ovarian insulin-like growth factor system. *Biology of Reproduction* 64: 1624-1632.
- Burke, S.M., Hampton, J.H., Staples, C.R., Thatcher, W.W.. 1998. Body condition maintenance of a persistent Influences first wave dominant follicle in dairy cattle. *Theriogenology* 49: 751-760.
- Burke, C.R.; Mussard, M.L., Grum, D.E., Day, M.L. 2001. Effects of maturity of the ovulatory follicle on induction potential of oestrus and ovulation in cattle with oestradiol benzoate. *Animal Reproduction Science* 66: 161-174.
- Beam, S.W., Butler, W.R. 1997. Energy balance and ovarian follicle prior to the first Development ovulation postpartum in dairy cows receiving three levels of dietary fat. *Biology of Reproduction* 56: 133-142.
- Butler, W.R. 2000. Nutritional interactions with reproductive performance in dairy cattle. *Animal Reproduction Science* 60-61: 449-457.
- Colazo, M.G., Kastelic, J.P., Small, J.A., Wilde, R.E., Ward, D.R., Mapletoft, R.J. 2007. Resynchronization of estrus in beef cattle: Ovarian function, estrus and fertility following progestin treatment and treatments to synchronize ovarian follicular development and estrus. *Canadian Veterinary Journal* 48: 49-56.
- Calvalho, J.B.P., Carvalho N.A.T., Reis, E.L., Nichi, M., Souza, A.H., Baruselli, P.S. 2008. Effect of early luteolysis in progesterone-base timed AI protocols in *Bos indicus* x *Bos taurus*, and *Bos taurus* heifers. *Theriogenology* 69: 167-175.
- Diskin, M.G., Mackey, D.R., Roche, J.F., Sreenan, J.M. 2003. Effects of nutrition and metabolic status on circulating hormone and ovarian follicle development in cattle. *Animal Reproduction Science* 78: 345-370.
- Delgado, R., Magana, J.G., Galina, C., Segura, J. 2004. Effect of body condition at calving and its changes during early lactation on postpartum reproductive performance of Zebu cows in a tropical environment. *Journal of Applied Animal Research* 26: 23-28.
- Díaz, G.S., Galina, C.S., Basurto, C.H., Ochoa, G.P. 2002. Effect of natural progesterone with or without the addition of estradiol benzoate on the occurrence of oestrus, ovulation and pregnancy in *Bos indicus* animals in tropical Mexico. *Archives of Veterinary Medicine* 34: 283-286

- Guzeloglu, A., Ambrose, J.D., Kassa, T., Diaz, T., Thatcher, M.J., Thatcher, W.W. 2001. Long-term follicular dynamics and biochemical characteristics of dairy cows in dominant follicles subject to acute heat stress. *Animal Reproduction Science* 66: 15-34.
- Hattab, I.N.C., Kadoom, A.K., Palme, R., Bamberg, E. 2000. Effect of estrus synchronization and Crestar on the relationship between concentrations of plasma and fecal progestagens in buffalo cows. *Theriogenology*. 54: 1007-1017.
- INEGI. 2005. Encyclopedia of the municipalities of Mexico, State of Yucatan. <http://www.e-local.gob.mx/work/templates/enciclo/yucatan/municipios/31006a.htm>.
- Kim, U.H., Suh, G.H., Nam, H.W., Kang, H.G., Kim, I.H. 2005. Follicular wave emergence, luteal function and synchrony of ovulation or estradiol benzoate following GnRH in CIDR-Treated, lactating Holstein cows. *Theriogenology* 63:260-268.
- Lucy, M.C., Staples, C.R., Michel, F.M., Thatcher, W.W. 1991. Energy balance and size and number of ovarian follicles detected by ultrasonography in early postpartum dairy cows. *Journal of Dairy Science* 74: 473-482.
- Lemaster, J.W., Yelich, J.V., Kempfer, J.R., Schrick, F.N. 1999. Ovulation and estrus characteristics in crossbred Brahman heifers treated with in an intravaginal progesterone-releasing insert in combination in prostaglandin F2 α and estradiol benzoate. *Journal of Animal Science* 77: 1860-1868.
- Medrano, E., Hernandez, O., Lamothe, C., Galina, C. 1996. Evidence of asynchrony in the onset of signs of estrous in Zebu cattle treated with a progestogen ear implant. *Research in Veterinary Sciences* 60: 51-54.
- Nogueira, G.P. 2004. Puberty in southamerican *Bos indicus* (Zebu) cattle. *Animal Reproduction Science* 82-83: 361-372.
- Peralta-Torres, J., Ake-Lopez, R., Centurion-Castro, F., Magaña-Monforte, J. 2010. Comparison of estradiol cypionate and estradiol benzoate effects on ovaric activity, estrus and ovulation on *Bos indicus* cows anestrous. *Journal of Animal and Veterinary Advances* 9: 466-470.
- Rasby, R.J., Wettemann, R.P., Harms, P.G., Lusby, K.S., Wagner, J.J. 1992. GnRH in the infundibular stalk-median eminence is related to percentage body fat in carcasses of beef cows. *Domestic Animals. Endocrinology* 9: 71-76.
- Ross, P.J., Aller, J.F., Callejas, S.S., Butler, H., Alberio, R.H. 2004. Estradiol benzoate given 0 or 24 h after the end of progestagen in postpartum suckled beef Treatment cows. *Theriogenology*, 62: 265-273.
- Rhodes, F.M., De'ath, G., Entwistle, K.W. 1995. Animal and temporal effects on ovarian follicular dynamics in Brahman heifers. *Animal Reproduction Science*. 38: 265-277.
- Rhodes, F.M., Entwistle, K.W., Kinder, J.E. 1996. Changes in ovarian function and gonadotropin secretion preceding the onset of nutritionally induced anestrous in *Bos indicus* heifers. *Biology of Reproduction* 55:1437-43.
- SAS. User's Guide. 2002. Statistics 8th ed. Cary (NC): SAS Institute Inc.
- Sartori, R., Fricke, P.M., Ferreira, J.C., Guinther, O.J, Wiltbank. 2001. Follicular deviation and adquisition of ovulatory capacity in bovine follicles. *Biology of Reproduction* 65: 1403-1409.
- Soto-Camargo, R., Galina, C.S., Rubio, G.I., Castille, G.E., Basurto, C.H. 1999. Effect of nutritional supplementation, body condition on estrus synchronization and mounting activity of Brahman heifers on pasture. *Revista de la Facultad de Agronomía (Luz)*. 16: 663-676.
- Sepúlveda, N.G.; Inostrosa, M.A., Peña, P., Risopatrón, J., Rodero, E. 2001. The onset of postpartum ovarian function in primiparous and multiparous dairy cows. *Archivos de Zootecnia* 50: 399-402.
- Sá Filho, M.F., Crespilho, A.M., Santos, J.E., Perri, G.A., Baruselli, P.S. 2010. Ovarian follicle diameter at timed insemination and estrous response Influence of ovulation and pregnancy likelihood after estrous synchronization with progesterone and progestin-based protocols in suckled *Bos indicus* cows. *Animal Reproduction Science* 120: 23-30

- Silva-Mena, C., Guzmán-Casas, R., Delgado-Leon, R., Ake-Lopez, R. 2002. Brahman heifers response to estrus synchronization with progestagen, sexual behavior and pregnancy rate. *Revista Biomédica* 13: 265-271.
- Velázquez, R.L., Fregoso, A.C., Lopez, R.W., Harris, C.J. 2011. Estrous response and conception rate in *Bos taurus*-*Bos indicus* postpartum cows, treated with bovine somatotropin ovulation induction in a program with progestogens and eCG. *Veterinaria México* 42: 245-251.
- Wiltbank, M.C.; Gumen, A., Sartori, R. 2002. Physiological classification of anovulatory conditions in cattle. *Theriogenology* 57: 21-52.
- White, F.J., Wettemann, R.P., Looper, M.L., Prado, T.M., Morgan, G.L. 2002. Seasonal effects on estrous and time of ovulation behavior in nonlactating beef cows. *Journal of Animal Science* 80: 3053-3059.

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