ISSN 2349-0837

Effect of recombinant bovine somatotropin on pregnancy rates of Nellore cows resynchronized with the use of new DIB® and third use, and inseminated at fixed-time

Josemara Silva Santos[1], Tânia Vasconcelos Cavalcante[2*], Francisca Elda Ferreira Dias[1], Domenica Palomaris Mariano de Souza[1], Alencariano J.S. Falcão[1], Mônica Arrivabene[2], Juliano Franco de Souza[7], José Adalmir Torres de Sousa[2], Viviane Mayumi Maruo[1]

[1]Universidade Federal do Tocantins, Programa de Pós Graduação em Ciência Animal Tropical, Escola de Medicina Veterinária e Zootecnia, campus de Araguaína, Tocantins, Brazil. BR 153, Km 112, CEP: 77.804-970. E-mail: josemara_ss@hotmail.com

[2]Universidade Federal do Piauí, Centro de Ciências Agrárias, Departamento de Clínica e Cirurgia Veterinária, campus Petrônio Portela, Teresina, Piauí, Brazil. E-mail: tania@ufpi.edu.br

[3]Universidade Federal do Tocantins, Escola de Medicina Veterinária e Zootecnia, campus de Araguaína, Tocantins, Brazil. E-mail: diasfef@gmail.com

[4] Universidade Federal do Tocantins, Campus Araguaína, Araguaína, Tocantins, Brazil. E-mail: domenica@uft.edu.br

[5]Universidade Federal do Tocantins, Escola de Medicina Veterinária e Zootecnia, campus de Araguaína, Tocantins, Brazil. E-mail: alencariano@uft.edu.br

[6]Universidade Federal do Piauí, Centro de Ciências Agrárias, Departamento de Clínica e Cirurgia Veterinária, campus Petrônio Portela, Teresina, Piauí, Brazil. E-mail: arrivabenevet@hotmail.com

[7]Laboratório Brio Genética e Biotecnologias Ltda, Araguaína, Tocantins, Brazil. E-mail: julianofranco@yahoo.com

[8]Universidade Federal do Piauí, Centro de Ciências Agrárias, Departamento de Clínica e Cirurgia Veterinária, campus Petrônio Portela, Teresina, Piauí, Brazil. E-mail: adalmir@ufpi.edu.br

[1]Universidade Federal do Tocantins, Programa de Pós Graduação em Ciência Animal Tropical, Escola de Medicina Veterinária e Zootecnia, campus de Araguaína, Tocantins, Brazil. E-mail: vivimaruo@uft.edu.br

ABSTRACT

The objective of the experiment was to evaluate the effects of recombinant bovine somatropin (rbST), and the reuse of the progesterone (P4) releasing devices in resynchronization, on the pregnancy rates of Nellore cows submitted to fixed-time artificial insemination. A group of 123 multiparae Nellore cows, was submitted to a resynchronization protocol: on day 0 a Bovine Intravaginal Device (DIB®) with 1,0g of P4 was implanted, associated with intramuscular administration of 2,0mg of estradiol benzoate (IM); on day 8 DIB was removed; and 1,0mg of estradiol cypionate, 0,15mg of prostaglandin $F_{2\alpha}$ and 300 UI of equine chorionic gonadotropin were administered; on day 10, fixed-time artificial insemination was conducted (FTAI). The cows were randomized into G1 (n=12) – without rbST / with used Bovine Intravaginal Device, G2 (n=50) – without rbST / with new DIB, G3 (n=11) - with rbST / with used DIB and G4 (n=50) – with rbST/ with new DIB. rbST was introduced on the eighth day of the protocol. Sixty days after TAI, pregnancy diagnoses were conducted, via rectal palpation. Blood samples were taken on day 0, 8 and 10 of the protocol, in order to assess P4 plasma concentrations. Pregnancy rates were statistically evaluated through Generalized Linear Models Theory and their significance was tested with Analysis of Deviance. Pregnancy rates were 58%, 40%, 81% and 48% for G1, G2, G3 and G4, respectively, with significant statistical difference for G3. Plasma concentrations of P4 were not statistically different among groups, or collections. In view of the results obtained, we concluded that the administration of rbST in association with P4 DIB, used for the third time, improves pregnancy rates. Estrus resynchronization and re-insemination positively impacted pregnancy rates.

Keywords: Cattle; growth hormone; progesterone; reproduction; zebu.

ISSN 2349-0837

INTRODUCTION

The growing demand for food in the world requires efficiency in beef cattle production. The profitability of the production system is closely related to the reproductive efficiency of the herd. The goal is to get a calf per cow per year [11]. However, that the best definition would be to have one calf per cow, with a twelve-month interval between births.

Beneficial effects in the performance are achieved through the use of recombinant bovine somatotropin (rbST) in fixed-time artificial insemination (FTAI) protocol [27].

Among the functions studied by several authors [6; 28; 31; 4] the action of rbST in the FTAI protocol is probably the one which best relates to the improvement of the pregnancy rates. As rbST enhances pituitary secretion of bST, as well as IGF-1 synthesis by the liver, it triggers a cascade of events that may directly or indirectly: accelerate *corpus luteum* (CL) growth and the secretion of progesterone (P4), during the luteal phase of the estrous cycle [31]; increase the population of small antral follicles; stimulate maturation of oocytes, and increase fertilization rates [27]; reduce serum concentrations of estradiol, around the 17th day of the estrous cycle in cattle; attenuate the production of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$); increase embryonic survival and development; and aid maternal recognition, thus increasing pregnancy rates [6].

Studies with rbST in beef cows are limited. The results observed in these studies are dissimilar due to the use of synchronization protocols, which differ in regard to the day of rbST administration [6; 28;1].

In cattle breeding programs, intravaginal P4-releasing devices are now widely known and used. They represent 50% or more of the costs of a synchronization program for FTAI [12]. A strategy to reduce the costs of these programs is the reuse of such devices.

In beginning, the devices were used only once and discarded. However, the reuse of these devices has demonstrated satisfactory efficiency without compromising pregnancy rates [9].

According to Pereira et al. (2011) 1g P4 devices (DIB®, Sincrogest®, Primer®, Cronipres®) began to be used up to three times with cattle, while 1,9g devices (CIDR®) are already being used four times in some properties. Dias et al. (2009) observed higher conception rates as the number of device uses increased.

P4 serum concentrations are almost undetectable until about 30 days postpartum. However, they demonstrate the activity of the *corpus luteum* when the concentration is higher than 1ng/ml. P4 serum profiles have been suggested to be a reliable method to objectively assess ovarian activity [16].

Some studies show that high concentrations of P4 can influence the pulsatility of luteinizing hormone (LH), which is responsible for the growth, maturation and ovulation of the dominant follicle (DF) [5].

According to Perez et al. (2009), the availability of exogenous P4 close to ovulation reduces the growth rate of the DF. Elevated P4 plasma concentrations (between 5 and 8ng/mL) result in low LH pulsatility, which is in line with other studies, that found that high serum concentrations of P4 decrease the ovulatory capacity of the DF, thus justifying the reuse of intravaginal devices.

Considering the mechanisms by which rbST can influence the reproductive physiology of cows, the efficiency of P4 reused devices, and several protocols proposed, with different results, the objective of the present experiment was to evaluate the effect of rbST and of reused P4 devices, in resynchronization, on the pregnancy rates of fixed-time inseminated Nellore cows.

MATERIAL AND METHODS

This study was authorized by the Ethics Committee on Animal Use of Universidade Federal do Tocantins, authorization procedure number 23101.003935/2012-57.

The experiment was conducted between the months of April and June 2012, at Maratá farm, in the municipality of Pio XII, state of Maranhão, Brazil. The property is located at 3°52'30" latitude S and 45°07'30" longitude W, with an annual average temperature of 27° C.

Clinically healthy multiparae females, suitable for reproduction, more than 90 days postpartum, aged 42-48 months, with mean body condition index of 3,0, in a scale of 1 to 5, were selected [15]. The animals were kept under extensive grazing conditions with Brachiaria Brizantha, water and mineral salt at will.

A group of 337 Nellore cows underwent a protocol for estrus synchronization, and then FTAI. After pregnancy diagnosis by ultrasonogram (30 days after FTAI), 123 cows, which were not pregnant, were selected and underwent a protocol of resynchronization and re-insemination.

This group of 123 cows was subjected to a protocol of estrus resynchronization and randomized into four groups: G1 (n = 12) - without rbST and reused DIB, G2 (n = 50) - without rbST and new DIB, G3 (n = 11) - with rbST and reused DIB, and G4 (n = 50) - with new DIB and rbST.

On day 8 of the estrus resynchronization protocol, 500 mg of rbST (Boostin®; Intervet - Schering Plough Animal Health) was administered. The estrus synchronization protocol was the same used in the resynchronization (Figure 1).

ISSN 2349-0837

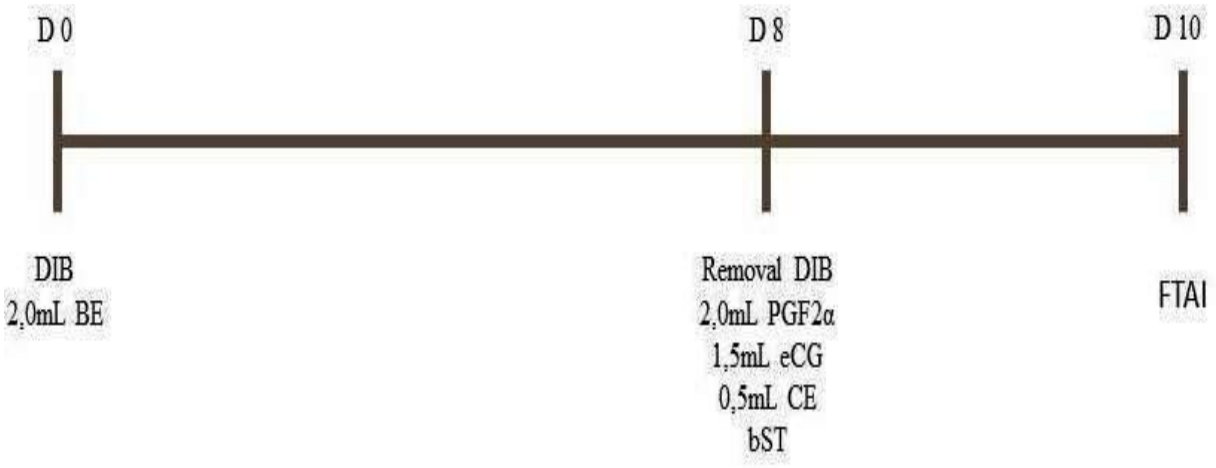

Figure 1 – Schematic representation of estrus synchronization and resynchronization protocols and ovulation induction.

The bovine intravaginal device impregnated with 1,0g P4 (DIB®, Schering-Plough Coopers) was implanted on day 0. Implantation was associated with the administration of 2,0mg of estradiol benzoate (Gonadiol®, Coopers Schering-Plough) intramuscularly (IM).

On day 8, the DIB® was removed, and 1,0mg of estradiol cypionate (ECP®, Pfizer), 0,15mg of prostaglandin $F_{2\alpha}$ (Veteglan® luteolitic, Hertape Calier) and 300 IU of equine chorionic gonadotropin (Folligon®, Schering Plough), were administered all IM.

On day 10, all the TAIs were performed by the same technician. The semen used had been obtained from an artificial insemination center, from seventeen breeding Nellore. Rectal palpation was used for pregnancy diagnosis, 60 days after FTAI.

To determine P4 serum concentrations, blood samples were taken from 10 animals in each group, on day zero, eight and ten of the resynchronization protocol. The blood was collected from the coccygeal vessels, into 10mL Vacutainer® tubes, without anticoagulant, and stored at a temperature between 5 and 8ºC, until they were centrifuged at 1422 x g for 20 minutes, in order to completely separate the serum, which was then stored in microtubes (*Eppendorf tubes*) and kept frozen at -18ºC for later analyses.

Serum P4 concentrations were obtained by using commercial kits (EIA Cayman Chemical Company, Ann Arbor, MI; EIA kits No. 0447860), as per manufacturer's instructions. The progesterone assay had a sensitivity of 6,5pg/ml, with a coefficient of variation of 4,4% intra-assay and 2,7% inter-assay.

Generalized Linear Models theory was used for the statistical analysis of pregnancy rates [18], and the significance of the effect of bSTr was tested through Deviance test Proc Logistic [29].

For P4 concentrations, the data structure is comprised of repeated measures over time, for each animal, within the groups. The data is analyzed by applying the repeated measurements technique using SAS Proc Mixed [17].

RESULTS AND DISCUSSION

Of a total of 337 cows submitted to the estrus synchronization protocol and fixed-time artificial insemination, 59,05 %(199) became pregnant. This percentage is within normal rates found in other studies [30; 23]. Just over 40% were not pregnant. In order to increase the rate of late or cumulative pregnancy, resynchronization of estrus was performed, thus providing these animals with a second chance of pregnancy within a breeding season.

The additional resynchronization costs are offset by the increase in the pregnancy rate [13]. The use of FTAI is essential for achieving success, as it enables the rationalization of labor and reduces losses, due to the failure in estrus detection [13; 12].

rbST was used with the aim of achieving better results in resynchronization, as it stimulates increased concentrations of GH and IGF-1, which exert positive effects on fertility [28].

And, seeking to increasingly lower the cost per pregnancy, the reuse of P4 devices was adopted, as they may correspond to more than half of the total cost per pregnancy [12; 9].

Pregnancy rates after resynchronization of estrus, using new DIB®, and in third use, with or without rbST treatment, were satisfactory (TABLE 1). This shows that the protocols adopted were effective for the resynchronization of ovulation, resulting in an average conception rate of 56%. Such rate is within the percentage normally found in FTAI studies, for beef cows with reused devices [24; 14; 25].

ISSN 2349-0837

Table 1 – Pregnancy rates of Nellore cows submitted toestrusresynchronization protocol, with new and reused P4 devices, with or without rbST administration.

Experimental Groups	Pregnancy Rates (%)
G1 –without rbST and with used DIB	58% (7/12)[b]
G2 –without rbST and with new DIB	40% (20/50)[b]
G3 –with rbST and with used DIB	81% (9/11)[a]
G4 –with rbST and with new DIB	48% (24/50)[b]

Averages followed by different lowercase letters in the column differ, as per Chi-square test (P<0,05).

The reuse of DIB® in the present study did not affect pregnancy rates, similarly to what was observed by Peixoto & Ulian Jr. (2007) and Chesta et al. (2005). This leads to the conclusion that there is sufficient residual P4 in these third use devices for synchronization of estrus and ovulation in thecows treated, as found by Pereira et al. (2011), Meneghetti et al. (2009) and Freitas et al. (2007). This shows that the reuse of other types of P4 devices does not compromise the reproductive efficiency of this category of animals.

Pregnancy rates in the groups treated with third use devices (G1 and G3) exceeded the others, especially G3, in which the result may be associated with the administration of rbST.

According to Rossetti et al. (2011), this hormone stimulates the concentrations of GH and IGF-1, which, in turn, accelerate embryonic development, on the eighth day after fertilization, through proliferation and/or cell differentiation [2]. They also modulate its metabolism to increase glucose transport [31], thus resulting in increased secretion of interferon-τ, at the time of maternal recognition of pregnancy [3]. Badinga et al. (2002) also found a potential of bST for inhibiting the synthesis of prostanoids, which may also be associated with improved embryo survival.

Plasma concentrations of P4 did not differ between groups or collection days (P<0,05) (Figure 2). Such P4 concentrations at the time of FTAI were lower than those reported by Perez et al. (2009), who concluded that concentrations of P4 between 5 and 8ng/ml at the time of ovulation, impairs LH pulsatility, and therefore, the diameter of the dominant follicle (DF) [8]. The positive correlations between the diameter of the ovulatory follicle and pregnancy rates are well-known, both being influenced by P4 concentrations [5].

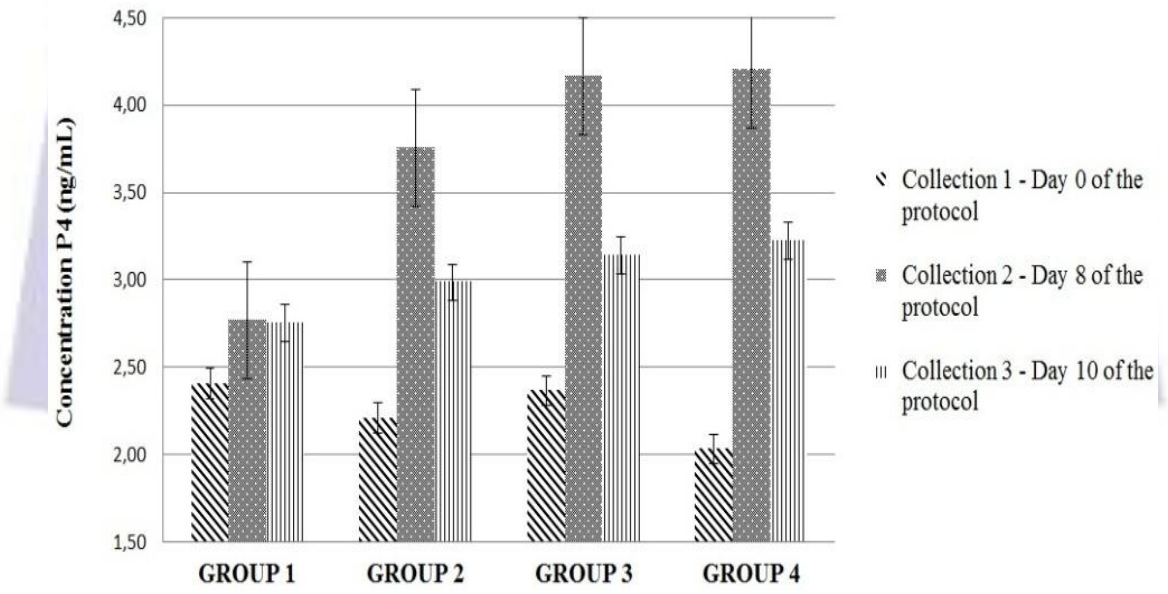

Figure 2 – Serum progesterone concentration in Nellore cows submitted to estrus resynchronization, collected in different days of the protocol. G1 without rbST and used DIB; G2 without rbST and new DIB; G3 with rbST used DIB; and G4 with rbST and new DIB (P <0.05).

After synchronization and resynchronization of estrus, a cumulative pregnancy rate of 76,8% (n = 259) was obtained, over a period of 45 days, without the need for observation of estrus, thus representing a gain for the producer (Figure 3).

ISSN 2349-0837

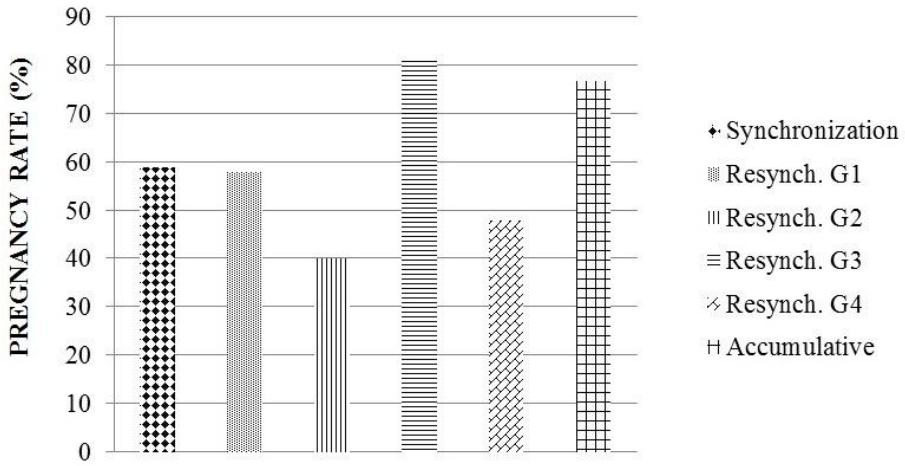

Figure 3 – Pregnancy rates in Nellore cows subjected to synchronization, resynchronization, and accumulated pregnancy rate.

Nowadays, obtaining acceptable conception rates after using estrus and ovulation synchronization protocols for FTAI has become a reality, however, not all synchronized cows become pregnant. That is the justification for the practice of providing a second chance to those cows, which that did not conceive after FTAI, in the same breeding season. This may result in a service rate of 100% [20].

Meneghetti et al. (2012), Nascimento (2009), Silva et al. (2007) and Penteado et al. (2005) also found results, which were similar to those presented here in.

CONCLUSION

The administration of rbST in multiparae Nellore cows, in a single dose, on day 8 of the resynchronization protocol, in association with P4 devices, reused for the third time, improved pregnancy rates.

Resynchronization of estrus and re-insemination exerted positive effects in the increase of cumulative pregnancy rates.

REFERENCES

1. [1]ALEXIO, M. A., KOZICKI, L. E., WEISS, R. R., SEGUI, M. S., PERCY JUNIOR, R. A. 2005. somatotropina recombinante bovina (bST) e a dinâmica folicular em bovinos leiteiros. Archives of Veterinary Science, 10(2):19-27.
2. [2]BADINGA, L., GUZELOGLU, A., THATCHER, W. W. 2002. Bovine somatotropin attenuates phorbol ester-induced prostaglandin F2*α* production in bovine endometrial cells. Journal Dairy Science, 85: 537–543.
3. [3] BELL, A. A.; HANSEN, P. J.; VRIES, A. 2009. Profitability somatotropin administration to increase first insemination conception rate in seasonal dairy herds with heat stress. Livestock Science, 126:38-45.
4. [4]BILBY, T. R., GUZELOGLU, A., KAMIMURA, S., PANCARCI, S. M., MICHEL, F., HEAD, H. H., THATCHER, W. W. 2004. Pregnancy and bovine somatotropin in nonlactating dairy cows: I. ovarian, conceptus, and insulin-like growth factor system responses. Journal Dairy Science, 87: 3256-3267.
5. [5]CARVALHO, 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-based fixed-time artificial insemination AI protocols in *Bos indicus*, *Bos indicus* x *Bos taurus* and *Bos taurus* heifers. Theriogenology, 69:167–175.
6. [6]CERÓN, J. H., AGUILAR, C. G. G. 2013. La somatotropina bovina recombinante y la reproducción em bovinos, ovinos y caprinos. Agrociencia, 47: 35-45.
7. [7]CHESTA, P., PINCINATO, D., PEÑA, D.M., PERES, L.C., TRÍBULO, R., BÓ, G.A. Efecto del tratamiento com DIB® de segundo o tercero uso em protocolos de resincronización de la ovulación y inseminación artificial a tiempo fijo. In: Simposio Internacional de Reproduccion Animal, 4, 2005, Córdoba. Anais..., Argentina, 2005.
8. [8]CIPRIANO, R. S., CARVALHO, B. A., MARANGONI, N. R., NOGUEIRA, G. P. 2011. LH and FSH concentration and follicular development in Nellore heifers submitted to fixed-time artificial insemination protocols with different progesterone concentrations. Animal Reproduction Science, 127:16–22.
9. [9]COLAZO, M. G., KASTELIC, J. P., WHITTAKER, P. R., GAVAGA, Q. A., WILDE, R., MAPLETOFT, R. J. 2004. Fertility in beef cattle given a new or previously used CIDR insert and estradiol, with or without progesterone. Animal Reproduction Science, 81: 25-34.

10. [10]DIAS, C. C., WECHSLER, F. S., DAY, M. L., VASCONCELOS, J. L. M. 2009. Progesterone concentrations, exogenous equine chorionic gonadotropin, and timing of prostaglandin F2α treatment affect fertility in postpuberal Nelore heifers. Theriogenology, 72:378-385.

11. [11]FERRAZ, H. T., VIU, M. A. O., LOPES, D. T. 2008. Sincronização da ovulação para realização da inseminação artificial em tempo fixo em bovinos de corte. Pubvet, .2(12).

12. [12]FREITAS, D. S., CHALHOUB, M., ALMEIDA, A. K. C., SILVA, A. A. B., SANTANA, R. C. M., RIBEIRO FILHO, A. L. 2007. Associação do diagnóstico precoce de prenhez a um protocolo de ressincronização do estro em vacas zebuínas. Revista Brasileira de Saúde e Produção Animal, 8(3): 170-117.

13. [13]GIRALDO, J. J. G. 2008. Sincronización y resincronización de celos y de ovulaciones em ganado de leche y carne. Revista Lasallista de Investigación. 5(2).

14. [14]GOTTSCHALL, C. S., BITTENCOURT, H. R., MATTOS, R. M., GREGORY, R. M. 2009. Antecipação da aplicação de prostaglandina em programa de inseminação artificial em tempo fixo em vacas de corte. Revista Brasileira de Saúde e Produção Animal, 10(4): 970-979.

15. [15]HOUGHTON, P. L., LEMENAGER, R. P., MOSS, G. E., HENDRIX, K. S. 1990. Prediction of postpartum beef cow body composition using weight to height ratio and visual body conditionscore. Journal of Animal Science, 68(5):1428-1437.

16. [16]KARA, N., BOUNECHADA, M., CHAIB, B. C. 2013. Effect of body condition score and parity on resumption of postpartum ovarian activity in montberliard dairy cows in Algerian Semi-Arid area. Journal of Animal Science Advances, 3(2):48-57.

17. [17]LITTELL, R. C., HENRY, P. R., AMMERMAN, C. B. 1998. Statistical analysis of repeated measures data using SAS procedures. Journal of Animal Science, 76:1216-1231.

18. [18]McCULLAGH, P.; NELDER, J. A. Generalized linear models, 2ª ed. Boca Raton: Chapman and Hall, 1989. 532p.

19. [19]MENEGHETTI, M., SÁ FILHO, O. G., PERES, R. F., LAMB, G. C., VASCONCELOS, J. L. 2009. Fixed-time artificial insemination with estradiol and progesterone for Bos indicus cows I: Basis for development of protocols. Theriogenology, 72: 210-218.

20. [20]MENEGHETTI, MARTINS JR, M. A.P., VILELA, E. R., LOSI, T. C., VASCONCELOS, J. L. M. Uso de Protocolo de IATF associado a diagnóstico precoce de gestação e ressincronização como estratégia para maximizar o número de vacas gestantes por IA em estação de monta reduzida. Disponível em http://lageado.com/artigo/6/0. Acesso 23 out 12.

21. [21]NASCIMENTO, V. A. Inseminação artificial em tempo fixo e transferência de embriões na eficiência reprodutiva de fêmeas bovinas. 160f. *Tese* apresentada à Universidade Federal de Viçosa. Viçosa, Minas Gerais. 2009.

22. [22]PEIXOTO JR., K. C. & ULIAN, C. M. V. 2007. Avaliação da taxa de prenhez de vacastratadas com dispositivos de progesteronareutilizados. Pubvet, 1(4).

23. [23]PENTEADO, L., SÁ FILHO, M. F., REIS, E. L., TORRES-JÚNIOR, J. R., MADUREIRA, E. H., BARUSELLI, P. S. Eficiência reprodutiva em vacas Nelore (*Bosindicus*) lactantes submetidas as diferentes manejos durante a estação de monta. *Anais...*XVI Reunião do Colégio Brasileiro de Reprodução Animal, 2005.

24. [24]PEREIRA, C. H., GOTTSCHAL, C. S., MATTOS, R. C., EVANGELISTA, G. T., SILVA, B. V. C., JOBIM, M. I. M., GREGORY, R. M. 2011. Luteólise antecipada em protocolo de inseminação artificial em tempo fixo com progesterona em vacas no pós-parto. Veterinária Notícias, 17(1):42-49.

25. [25]PERES, R. F. G. Efeito da concentraçãopré e pós-ovulatória de progesterona em protocolos de IATF em fêmeas Nelore. 87f. Dissertação (Mestrado) Faculdade de Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu, 2008.

26. [26]PEREZ, R. F. G., CLARO JÚNIOR, I., SÁ FILHO, O. G., NOGUEIRA, G. P., VASCONCELOS, J. L. M. 2009. Strategies to improve fertility in *Bos indicus* postpubertal heifers and nonlactating cows submitted to fixed-time artificial insemination. Theriogenology, 72:681-689.

27. [27]ROSAS, L. E. V., AGUAYO, C. F., ORDAZ, R. L., CERÓN, J. H. 2011. Respuesta estral y porcentaje de concepción en vacas *Bos taurus-Bos indicus* pos-parto, tratadas com la hormona bovina Del crecimiento e nun programa de inducción de La ovulación com progestágenos y eCG.Veterinária México, 42(3).

28. [28]ROSSETTI, R. C., PERDIGÃO, A., MESQUITA, F. S., SÁ FILHO, M., NOGUEIRA, G. P., MACHADO, R., MEMBRIVE, C. M. B., BINELLI, M. 2011. Effects of flunixin meglumine recombinant bovine somatotropin and/or human chorionic gonadotropin on pregnancy rates in Nelore cows. Theriogenology, 76:751-758.

29. [29]SAS – Satistical Analyses System. SAS/STAT User's guide, version 8, v. 2, 2001.

ISSN 2349-0837

30. [30]SILVA, A. S., SILVA, E. V. da C., NOGUEIRA, E., ZÚCCARI, C. E. S. N. 2007. Avaliação do custo/benefício da inseminação artificial convencional e em tempo fixo de fêmeas bovinas pluríparas de corte. Revista Brasileira de Reprodução Animal. Belo Horizonte, 31(4): 443-455.
31. [31]STARBUCK, M. J., INSKEEP, E. K., DAILEY, R. A. 2006. Effect of a single growth hormone (rbST) treatment at breeding on conception rates and pregnancy retention in dairy and beef cattle. Animal Reproduction Science, 93:349–359.