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Synchronization techniques to increase the utilization of artificial insemination in beef and dairy cattle

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Abstract

The main objective of the implementation of Artificial Insemination (AI) in cattle is to produce a sustained genetic progress in the herd. Although AI is an old reproductive biotechnology, its widespread implementation is very recent and is mainly due to the use of protocols that allows the AI without heat detection, commonly called fixed-time artificial insemination (FTAI). The development of FTAI protocols also allowed the application of AI in larger, extensively managed, herds and especially in suckled cows instead of just reducing the breeding programs to the heifers. FTAI treatments are widely used in South America, with about 2,500,000 cows inseminated in the last season in Argentina and about 6,500,000 in Brazil. This manuscript aims to present and describe several treatments available and some of the factors that may affect pregnancy rates.

Keywords: eCG, estradiol, fixed-time AI, GnRH, progestins.

Introduction

Artificial Insemination has been used widely to reproduce the most valuable genetics. However, factors such as nutrition, management, and estrus detection efficiency affect the widespread use of this technology in most cattle operations. The most useful alternative to increase the number cows inseminated is to apply protocols that allow for AI without the need for estrus detection, usually referred to as fixed-time AI (FTAI). The objective of this manuscript is to review the available protocols that synchronize ovulation in beef and dairy cattle, making a special emphasis in those currently used in South-America.

Synchronization treatments in beef cattle

Estradiol and progestin treatments have been widely used over the past several years in estrus synchronization programs in cattle (Macmillan and Burke, 1996; Bó and Baruselli, 2002) and are the preferred treatment for FTAI of beef cattle in South America. Treatments consist of insertion of a progestin-releasing device and the administration of estradiol on day 0 (to synchronize follicular wave emergence), PGF at the time of device removal on days 7, 8 or 9 (to ensure luteolysis), and the subsequent application of a lower dose of estradiol 24 h later or GnRH/LH 48 to 54 h later to synchronize ovulation (Bó *et al*., 2002a, b; Martinez *et al*., 2002a). The pregnancy per AI (P/AI) reported with these protocols has been between 40 to 50%, ranging from 27.8 to 75.0%. The factors that most affected pregnancy rates were body condition score (BCS) and cyclicity of the cows (Bó *et al*., 2002b; 2007).

The application of equine Chorionic Gonadotropin (eCG) at the time of removal of a progestin device has been extensively used in *Bos indicus* herds (reviewed in Baruselli *et al*., 2004) and in *Bos taurus* herds with high incidence of postpartum anestrus (Bó *et al*., 2002b). Probably the most important effect of eCG is the stimulation of the growth of the dominant follicle that consequently increases ovulation rate (Sá Filho *et al*., 2010), especially in cows in postpartum anestrus and/or in low BCS (Bó *et al*., 2002b; 2007). Analysis from 9,668 FTAI has shown that animals treated with progestin-devices must have a BCS higher than 2.5 (scale 1 to 5) and ideally >3 to achieve pregnancy rates of 50% or higher (Bó *et al*., 2007). Conversely, the addition of eCG allowed for pregnancy rates close to 50% in cows with a BCS of ≤2.5 (Bó *et al*., 2007). It is very important to note that these results have been achieved only when cows were gaining body condition during the breeding season. If drought conditions or lack of feed prevent cattle from improving body condition during the breeding season, pregnancy rates will most probably be 35% or less, even after the administration of eCG (Bó *et al*., 2007).

Restricted suckling or calf removal associated with progestin devices has also been used for the induction of cyclicity in beef cows (Williams *et al*., 2002). However the response to temporary weaning (TW) seems to be related to body condition of the cows. Two experiments were conducted to compare the effects of eCG treatment and TW on ovulation and pregnancy rates in postpartum cows in moderate to low body condition and only 22% of them with a CL (Bó *et al*.,

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2007). We found that both TW and eCG increased ovulation rates [(TW = 7/10, 70.0%; eCG = 12/20, 60.0%; and control cows (no TW or eCG treatment: 2/9, 22.2%)]. Although the growth rate of the ovulatory follicle was greater ($P < 0.05$) in cows treated with eCG (1.1 ± 0.1 mm/day) than in those not treated with eCG (0.6 ± 0.1 mm/day), the ovulatory follicle was smaller in TW cows (9.9 ± 0.4 mm), compared to those not TW (11.8 ± 0.3 mm; $P < 0.05$). P/AI were increased in cows treated with eCG (eCG, 154/377, 40.8% *vs*. no eCG, 128/392, 32.6%; $P < 0.01$); whereas no differences were found between cows that were TW (141/379, 37.2%) and those that were not (141/390, 36.1%; $P > 0.7$). There was an interaction between TW and pregnancy rates that was due to an improvement in P/AI when cows had >2.5 BCS (TW: 48.3% *vs*. no TW: 28.2; $P < 0.05$) and not improvement when cows were ≤2.5 BCS (36.4 *vs*. 37.9%). It was concluded that the use of eCG but not TW improved P/AI following FTAI in postpartum *Bos indicus* x *Bos taurus* crossbred cows in moderate to low body condition. Results also suggest that the eCG-related increase in P/AI may be due to the final growth rate of the ovulatory follicle. On the other hand, the absence or little effect of TW on pregnancy rates contrasts with data from other studies done with Nelore cows (Penteado *et al*., 2004; Sá Filho *et al*., 2009). Therefore, the beneficial effects of TW may differ, depending on the management and BCS of the cows. Moreover, to set up a TW program creates logistical problems in several farms, especially in medium to small farms. Nevertheless, the results from both studies confirmed those reported previously that eCG increased P/AI in suckled anestrous cows enrolled in a FTAI program utilizing progestin devices and estradiol (Bó *et al*., 2002b, 2007; Baruselli *et al*., 2004).

GnRH-based treatment protocols have been used in North-America for FTAI in beef cattle (Geary *et al*., 2001). These treatments have also been used in FTAI programs in *Bos indicus* cattle (Barros *et al*., 2000; Williams *et al*., 2002; Baruselli *et al*., 2004). However, overall pregnancy rates (Baruselli *et al*., 2004; Saldarriaga *et al*., 2005) have often been lower than those rates reported in *Bos taurus* cattle, with low conception rates in anestrous cows (Fernandes *et al*., 2001; Baruselli *et al*., 2004). The addition of a progestin-releasing device increased pregnancy rates in anestrous *Bos taurus* cows (Lamb *et al*., 2001); however, this treatment has not been widely used in South-America for FTAI of beef cows and heifers. Conception rates have been often lower than 50% in *Bos indicus* cattle (Saldarriaga *et al*., 2005; Pincinato, 2012). The addition of eCG has shown an improvement in P/AI in *Bos indicus* cows in postpartum anestrous (Pincinato, 2012) and in *Bos taurus* primiparous cows (Small *et al*., 2009); but no improvement in P/AI have been reported in *Bos taurus* cows in good BCS (5.7 ± 0.71 BCS in the 1 to 9 scale; Marquezzini *et al*., 2013).

Fixed-time AI treatments for dairy cattle

In dairy cattle, FTAI protocols currently used in South-America are based on estradiol and progestin and/or based on GnRH. Estradiol and progestin treatments for dairy cattle are similar to those previously described for beef cattle, with reported P/AI between 35 to 55% (Bó *et al*., 2009).

Ovsynch protocols have been used extensively in recent years for FTAI in dairy cattle in USA (Caraviello *et al*., 2006). The protocol currently used in dairy cattle consists of an injection of GnRH followed by PGF 7 days later, a second injection of GnRH 56 h after PGF treatment with fixed-time AI 16 h later (Brusveen *et al*., 2008). The rationale for this protocol is that the first GnRH will induce LH release and ovulation of a dominant follicle and emergence of a new follicular wave within 2 days. The administration of PGF 7 days later will induce luteolysis, and the second GnRH will induce LH release synchronizing ovulation of the new dominant follicle (Pursley *et al*., 1995).

Recent studies have shown that the first GnRH resulted in ovulation in 44 to 54% of dairy cows (Bello *et al*., 2006; Colazo *et al*., 2009), 56% of beef heifers (Martinez *et al*., 1999), and 60% of beef cows (Small *et al*., 2009), and the emergence of a new follicular wave was synchronized only when treatment caused ovulation (Martinez *et al*., 1999). If the first GnRH does not synchronize follicular wave emergence, ovulation following the second GnRH may be poorly synchronized (Martinez *et al*., 2002b). Prevention of the early ovulations by addition of a progestin-releasing device to a 7-day GnRH-based protocol has improved pregnancy rates in heifers after fixed-time AI (Martinez *et al*., 2002b). Another approach to increase the number of cows responding to a GnRH-based protocol is to pre-synchronize estrus with two PGF treatments 14 days apart, with the last PGF given 10 to 12 days before the first GnRH (Moreira *et al*., 2001; Galvão *et al*., 2007). This protocol has been named Pre-Synch Ovsynch and has been shown to improve pregnancy rates in GnRH-based FTAI protocols in dairy cows.

The Ovsynch protocol has not been successfully used to synchronize cows in postpartum anestrus. This protocol appears to induce ovulation in a high percentage of anestrous dairy cows, but some of these cows have a subsequent short luteal phase (Gumen *et al*., 2003; McDougall, 2010) resulting in lower conception rates than in cycling cows (Moreira *et al*., 2001). Thus, although Ovsynch may induce ovulation in non-cycling cows, there is still likely to be a reduction in conception rates in these cows. One alternative treatment for cows in post-partum anestrus is to combine a progesterone releasing device with the Ovsynch protocol. Although an initial experiment done with high producing cows in North-America showed a significant improvement in P/AI (55.2 *vs*. 34.7%) for cows treated or not treated with progesterone releasing devices at the time of the first GnRH (Pursley *et al*., 2001), another review (Stevenson *et al*., 2006) has shown that the results are surprisingly variable, and are probably related to the high progesterone clearance rate of the North-American high producing dairy cows managed in intensive systems (Wiltbank *et al*., 2006). In a recent study, the addition of two CIDR devices increased pregnancy rates in cows in anovulatory anestrus (Bisinotto *et al*., 2013). Another approach to increase progesterone concentrations during Ovsynch in North-America includes the administration of GnRH 6 or 7 days prior Ovsynch. These treatments are called Double Ovsynch (Souza *et al*., 2008) or G6G (Bello *et al*., 2006) and have been reported to increase P/AI. Nevertheless, in pasture-based dairy cows, in which liver progesterone clearance is not that high, the insertion of a progesterone-releasing device had resulted in significant improvements in P/AI (Veneranda *et al*., 2008; McDougall, 2010).

Equine Chorionic Gonadotropin (eCG) in dairy cattle

Contrasting results have been reported about the use of 400 IU of eCG at the time of removal of a progestin device in dairy cattle. In three experiments performed with lactating dairy cows in Argentina, P/AI were significantly higher (P < 0.01) in cows treated with progesterone-releasing devices, estradiol and eCG (145/298; 48.7%) than in those treated with the Ovsynch protocol plus progesterone-releasing devices (117/298; 39.3%; Veneranda *et al*., 2006, 2008). Furthermore, the treatment of lactating dairy cows with eCG in Brazil has shown differences in pregnancy rates among cows with lower body condition score (BCS <2.75; Souza *et al*., 2009). They were higher in those treated with eCG (38.0%; 30/79) than in those not treated with eCG (15.2%; 12/79; P < 0.05). Conversely, pregnancy rates did not differ in cows with BCS >2.75 and treated (29.9%; 93/311) or not treated (33.1%; 100/302) with eCG (Souza *et al*., 2009). However, in a more recent study, adding either 400 or 600 IU eCG to FTAI protocols was inefficient to alter follicular and luteal dynamics and increase P/AI in high producing dairy cows that were more than 150 days in milk in Brazil (Ferreira *et al*., 2013).

An experiment was performed in New Zealand to compare the reproductive response of lactating, seasonally calving, dairy cows diagnosed by rectal palpation with anovulatory anestrus (Bryan *et al*., 2010). In the first experiment, the addition of eCG to an estradiol and progestin treatment resulted in increased P/AI (eCG: 48.9%; n = 432 *vs*. no eCG: 43.1%; n = 420; P = 0.059), especially in cows older than 5 years of age.

A follow-up study was designed to evaluate the effect of adding eCG to a GnRH-based synchronization programs in New Zealand (Bryan *et al*., 2013). Cows of 15 commercial dairy farms (n = 1991) were selected for inclusion on the basis of non-observed estrus by 7 days prior to the planned start of mating (PSM) and diagnosed with anovulatory anestrus by rectal palpation at the beginning of the trial. Cows were included for treatment according to the trial protocol (2 x 2 factorial). On day 0, all cows received a Cue-Mate intravaginal device (Bioniche Animal Health, Australasia) and an injection of 100 µg gonadorelin (GnRH; 1 ml Ovurelin, Bayer, New Zealand) and were randomly assigned to have their device removed and receive 500 µg of cloprostenol (Ovuprost, Bayer) on day 6 or 7. Within each group cows were further subdivided to receive 400 IU of eCG (Pregnecol, Bioniche) or no eCG (controls) at device removal. All cows were AI to detected heats and if not detected in heat, cows received a second injection of GnRH between 52 and 70 h after Cue-Mate device removal and were FTAI 72 h after device pull. Primary outcomes considered were 7-day in calf rate (ICR); 28-day ICR; and days to conception (DTC). There were no significant differences between a 6- or 7-day program, and there were no 6/7-day program by eCG interactions. However, inclusion of eCG into either a 6 or 7 day GnRH and Cue-Mate synchronization program increased 7 day ICR (P < 0.046), 28 day ICR (P < 0.008), and decreased median days to conception (P < 0.005). Overall, treatment with eCG increased 28 day ICR from 50.4 to 56.2%. These results confirm that the addition of eCG into synchronization protocols for anestrous cows in seasonally calving herds has a significant value to get more cows pregnant at the beginning of the breeding season.

Two follow-up experiments were conducted in Argentina, to evaluate the effect of adding eCG to GnRH plus progesterone protocol on follicular dynamics and pregnancy rates in lactating Holstein cows in a mixed management system (i.e. 35% pasture and 65% grain and silage). In experiment 1, 40 Holstein cows with 65.0 ± 3.6 days in lactation, BCS of 2.9 ± 0.1 (scale of 1 to 5), and producing 32.3 ± 3.1 l of milk were used. On day 0, all cows received a Cue-Mate device and 0.05 mg lecirelin (GnRH, Biosyn-OV, Biotay, Argentina). On day 6, cows were divided into two groups to receive 0.15 mg of D (+) cloprostenol (PGF, Bioprost-D, Biotay) at the time of removal of the Cue-Mate device (Group 6 days) or 24 h after (Group 7 days). Furthermore, each group was subdivided (2 x 2 factorial) to receive 400 IU eCG (Pregnecol, Bioniche) upon removal of the Cue-Mate or no other treatment at that time. All cows received a second GnRH 56 h after Cue-Mate removal and were FTAI at 72 h after Cue-Mate pull. The cows were examined by transrectal ultrasonography to determine ovulation rate to the first GnRH and to the second GnRH. On day 0, 32/40 of the cows had a CL. Ovulation to the first GnRH was 75% (30/40), with no differences between groups (P = 0.45). There were no differences between cows treated or not with eCG on the characteristics of the ovulatory follicle (17.1 ± 0.4 mm *vs*. 16.2 ± 0.5 mm; P > 0.14) and plasma progesterone concentrations in the ensuing luteal phase (7.6 ± 0.5 *vs*. 7.1 ± 0.5 ng/ml progesterone, P > 0.6). In experiment 2, 453 lactating cows were synchronized with the same treatments evaluated in experiment 1. P/AI were higher in cows treated with Cue-Mates for 7 days (86/227, 38%) than those treated for 6 days (61/226, 27%; P < 0.01). However, there were no differences between the cows treated (75/227; 33%) or not treated (72/226, 32%; P > 0.8) with eCG. Collectively, the results from these studies show that the addition of eCG does not increase pregnancy rates in Holstein cows treated with Ovsynch, suggesting that the addition of eCG would increase P/AI only in cows which are non-cycling and/or nutritionally restricted and with only small follicles that would fail to ovulate after the first and/or second GnRH. Higher producing dairy cows, which usually have large size follicles, may not be benefited by the extra stimulation of the growth rate of the dominant follicle induced by eCG.

The 5-day Cosynch protocol

Bridges *et al*. (2008) compared a 7-day Cosynch protocol plus progestin device with FTAI at 60 h and a 5-day Cosynch protocol with FTAI at 72 h in postpartum beef cows. In that study, P/AI was 11% higher with the 5-day protocol. Santos *et al*. (2010) reported similar findings in dairy cattle. The hypothesis proposed was that the 5-day protocol provided for a longer proestrus with increasing estradiol concentrations due to continuous gonadotropin support for the dominant follicle. The ovulatory follicle of cows in the 5-day program benefited from this extra time and additional gonadotropin support. However, due to a shorter interval between the first GnRH and induction of luteolysis in the 5-day protocol, two injections of PGF 6 to 24 h apart were necessary to induce complete regression of the GnRH-induced CL.

More recently, Kasimanickam *et al*. (2012) reported that heifers inseminated at 56 h in a 5-day Cosynch protocol had, on average, a 10.3% higher P/AI than heifers inseminated at 72 h. In addition, Colazo *et al*. (2011) showed that P/AI did not differ between a 5-day and 7-day Cosynch protocols with a single administration of PGF in dairy heifers. In that study, the use of the first GnRH in the 5-day Cosynch protocol also did not seem to be necessary as P/AI did not differ when it was not used. Conversely, Lima *et al*. (2011) observed an increased P/AI in dairy heifers receiving the final GnRH concurrent with AI at 72 h after PGF compared to 16 h before AI. However, they also showed no benefit of a first GnRH. In afollow-up study, P/AI were greater in heifers receiving GnRH at CIDR insertion, but only when two PGF were administered at CIDR removal and 24 h later.

We recently carried out an experiment to evaluate a new treatment based on estradiol but with a prolonged proestrus (de la Mata and Bó, 2012). In the first study 28 *Bos taurus* beef heifers, that were 16 and 17 months of age were randomly divided into two groups. Heifers in Group 1 (EB 6-day, n = 14) received 2 mg EB and an intravaginal device with 0.6 g of progesterone (Emefur 0.6 g, Merial Argentina SA) whereas those in Group 2 (n = 14) were treated with the 5-day Cosynch. All heifers received 150 µg of D-cloprostenol (Emefur, Merial) at device removal and were FTAI and received GnRH 72 h later. All heifers were examined by ultrasonography to monitor follicular development and ovulation. The initiation of a new follicular wave occurred earlier (P < 0.05) in heifers treated with GnRH (2.1 ± 1.0 days) than in those treated with EB (3.7± 0.9 days). However, ovulation rate (91.6 *vs*. 92.8%), the diameter of the ovulatory follicle (11.7 ± 0.2 mm *vs*. 12.0 ± 0.5 mm), the interval from PGF to ovulation (97.1 ± 17.4 h *vs*. 95.1 ± 12.5 h) and P/AI (50.0 *vs*. 57.1%) did not differ (P > 0.5) between heifers in the EB 6-d group and those in the 5-day Cosynch group. This treatment is still under investigation but in field trials done with 854 commercial beef heifers, overall P/AI with the EB 6-d treatment averaged 53.7% (range: 35 to 71.8%). In another follow-up study with Holstein heifers that is currently underway, preliminary P/AI were 59.2% (29/49) for the EB-6 d, 53.3% (24/45) for the 5-day Cosynch and 47.9% (23/48; P = 0.3) for those in the control group, which were treated with the standard EB plus progestin protocol for 7 days. In conclusion, these treatments proved to be efficient for synchronizing ovulation in heifers. However, it is necessary to perform more studies with a large number of animals to determine if these treatments increase P/AI compared to the traditional EB plus progestin treatment currently used by most practitioners in South-America.

Summary and conclusions

The use of protocols that control follicular development and ovulation has the advantage of being able to inseminate cows without the need for detecting estrus. These treatments have been shown to be practical and easy to perform by the farm staff, and more importantly, they do not depend on the accuracy in estrus detection. Treatments with GnRH and progestin-releasing devices and estradiol have provided for FTAI in beef and dairy cattle and the addition of eCG have been especially useful in increasing pregnancy rates in cows experiencing post-partum anestrus. Finally, shorter synchronization treatments that provide for a longer proestrus are an interesting new alternative for FTAI; however, more studies are needed to determine if these treatments increase P/AI compared to the traditional EB plus progestin treatment currently used by most practitioners in South-America.

References

Barros CM, Moreira, MBB, Figueiredo RA, Teixeira AB, Trinca LA. 2000. Synchronization of ovulation in beef cows (*Bos indicus*), using GnRH, PGF2a and estradiol benzoate. *Theriogenology*, 53:1121-1134.

Baruselli PS, Reis EL, Marques MO, Nasser LF, Bó GA. 2004. The use of treatments to improve reproductive performance of anestrus beef cattle in tropical climates. *Anim Reprod Sci*, 82/83:479-486.

Bello NM, Steibel JP, Pursley JR. 2006. Optimizing ovulation to first GnRH improved outcomes to each hormonal injection of Ovsynch in lactating dairy cows. *J Dairy Sci*, 89:3413-3424.

Bisinotto RS, Ribeiro ES, Lima FS, Martinez N, Greco LF, Barbosa LFSP, Bueno PP, Scagion LFS, Thatcher WW, Santos JEP. 2013. Targeted progesterone supplementation improves fertility in lactating dairy cows without a corpus luteum at the initiation of the timed artificial insemination protocol. *J Dairy Sci*, 96:2214-2225.

Bó GA, Baruselli PS. 2002. Programas de inseminación artificial a tiempo fijo en el ganado bovino en regiones subtropicales y tropicales. *In*: Gonzalez-Stagnaro C, Soto Belloso E, Ramirez Iglesia L (Ed.). *Avances en la Ganadería Doble Propósito*. Maracaibo, Venezuela: Fundación GIRARZ, Ed Astro Data. pp. 497-514.

Bó GA, Cutaia L, Tribulo R. 2002a. Tratamientos hormonales para inseminación artificial a tiempo fijo en bovinos para carne: algunas experiencias realizadas en Argentina. Primera parte. *Taurus*, 14:10-21.

Bó GA, Cutaia L, Tribulo R. 2002b. Tratamientos hormonales para inseminación artificial a tiempo fijo en bovinos para carne: algunas experiencias realizadas en Argentina. Segunda parte. *Taurus*, 15:17-32.

Bó GA, Cutaia L, Peres LC, Pincinato D, Maraña D, Baruselli PS. 2007. Technologies for fixed-time artificial insemination and their influence on reproductive performance of *Bos indicus* cattle. *In*: Juengel JL, Murray JF, Smith MF (Ed.). *Reproduction in Domestic Ruminants VI*. Nottingham, UK: Nottingham University Press. pp. 223-236.

Bó GA, Cutaia LE, Souza AH, Baruselli PS. 2009. Actualización sobre protocolos de IATF en bovinos de leche utilizando dispositivos con progesterona. *Taurus*, 41:20-34.

Bridges GA, Helser LA, Grum DE, Mussard ML, Gasser CL, Day ML. 2008. Decreasing the interval between GnRH and PGF2α from 7 to 5 days and lengthening proestrus increases timed-AI pregnancy rates in beef cows. *Theriogenology*, 69:843-851.

Brusveen DJ, Cunha AP, Silva CD, Cunha PM, Sterry RA, Silva EPB, Guenther JN, Wiltbank MC. 2008. Altering the time of the second gonadotropin-releasing hormone injection and artificial insemination (AI) during ovsynch affects pregnancies per AI in lactating dairy cows. *J Dairy Sci*, 91:1044-1052.

Bryan MA, Bó GA, Heuer C, Emslie FR. 2010. Use of equine chorionic gonadotrophin in synchronised AI of seasonal-breeding, pasture-based, anoestrous dairy cattle. *Reprod Fertil Dev*, 22:126-131.

Bryan MA, Bó G, Mapletoft RJ, Emslie FR. 2013. The use of equine chorionic gonadotropin in the treatment of anestrous dairy cows in gonadotropin-releasing hormone/progesterone protocols of 6 or 7 days. *J Dairy Sci*, 96:122-131.

Caraviello DZ, Weigel KA, Fricke PM, Wiltbank MC, Florent MJ, Cook NB. 2006. Survey of management practices related to the reproductive performance of dairy cattle on large commercial farms in the United States. *J Dairy Sci*, 89:4723-4735.

Colazo MG, Gordon MB, Rajamahendran R, Mapletoft RJ, Ambrose DJ. 2009. Pregnancy rates to timed artificial insemination in dairy cows treated with gonadotropin-releasing hormone or porcine luteinizing hormone. *Theriogenology*, 72:262-270.

Colazo MG, Ambrose DJ. 2011. Neither duration of progesterone insert nor initial GnRH treatment affected pregnancy per timed-insemination in dairy heifers subjected to a Co-synch protocol. *Theriogenology*, 76:578-588.

de la Mata JJ, Bó GA. 2012. Sincronización de celos y ovulación utilizando protocolos con benzoato de estradiol y GnRH en períodos reducidos de inserción de un dispositivo con progesterona en vaquillonas para carne. *Taurus*, 55:17-23.

Fernandes P, Teixeria AB, Crocci AJ, Barros CM. 2001. Timed artificial insemination in beef cattle using GnRH agonist, PGF2a and estradiol benzoate (EB). *Theriogenology*, 55:1521-1532.

Ferreira RM, Ayres H, Sales JNS, Souza AH, Rodrigues CA, Baruselli PS. 2013. Effect of different doses of equine chorionic gonadotropin on follicular and luteal dynamics and P/AI of high-producing Holstein cows. *Anim Reprod Sci.* (in press).

Galvão KN, Sá Filho MF, Santos JEP. 2007. Reducing the interval from presynchronization to initiation of timed artificial insemination improves fertility in dairy cows. *J Dairy Sci*, 90:4212-4218.

Geary TW, Whittier JC, Hallford DM, MacNeil MD. 2001. Calf removal improves conception rates to the Ovsynch and Co-synch protocols. *J Anim Sci*, 79:1-4.

Gumen A, Guenther JN, Wiltbank MC. 2003. Follicular size and response to Ovsynch versus detection of estrus in anovular and ovular lactating dairy cows. *J Dairy Sci*, 86:3184-3194.

Kasimanickam R, Asay M, Firth P, Whittier WD, Hall JB. 2012. Artificial insemination at 56 h after intravaginal progesterone device removal improved AI pregnancy rate in beef heifers synchronized with five-day CO-Synch controlled internal drug release (CIDR) protocol. *Theriogenology*, 77:1624-1631.

Lamb GC, Stevenson JS, Kesler DJ, Garverick HA, Brown DR, Salfen BE. 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2α for ovulation control in postpartum suckled beef cows. *J Anim Sci*, 79:2253-2259.

Lima FS, Ayres H, Favoreto MG, Bisinotto RS, Greco LF, Ribeiro ES, Baruselli PS, Risco CA,

Thatcher WW, Santos JEP. 2011. Effects of gonadotropin releasing hormone at initiation of the 5-day timed artificial insemination (AI) program and timing of induction of ovulation relative to AI on ovarian dynamics and fertility of dairy heifers. *J Dairy Sci*, 94:4997-5004.

Macmillan KL, Burke CR. 1996. Effects of oestrus cycle control on reproductive efficiency. *Anim Reprod Sci*, 42:307-320.

Marquezini GHL, Mercadante VRG, Olson KC, Jaeger JR, Perry GA, Stevenson JS, Lamb GC. 2013. Effects of equine chorionic gonadotropin on follicle development and pregnancy rates in suckled beef cows with or without calf removal. *J Anim Sci*, 91:1216-1224.

Martinez MF, Adams GP, Bergfelt D, Kastelic JP, Mapletoft RJ. 1999. Effect of LH or GnRH on the dominant follicle of the first follicular wave in heifers. *Anim Reprod Sci*, 57:23-33.

Martinez MF, Kastelic JP, Adams GP, Cook RB, Olson WO, Mapletoft RJ. 2002a. The use of progestins in regimens for fixed-time artificial insemination in beef cattle. *Theriogenology*, 57:1049-1059.

Martinez MF, Kastelic JP, Adams GP, Mapletoft RJ. 2002b. The use of a progesterone-releasing device (CIDR) or melengestrol acetate with GnRH, LH or estradiol benzoate for fixed-time AI in beef heifers. *J Anim Sci*, 80:1746-1751.

McDougall S. 2010. Effects of treatment of anestrous dairy cows with gonadotrophin-releasing hormone, prostaglandin and progesterone. *J Dairy Sci,* 93:1944-1959.

Moreira F, Orlandi C, Risco CA, Schouten, MJ, Lopes F, Thatcher WW. 2001 Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. *J Dairy Sci*, 84:1646-1659.

Penteado L, Ayres H, Madureira EH, Baruselli PS. 2004. Efeito do desmame temporário na taxa de prenhez de vacas Nelore lactantes inseminadas em tempo fixo. *Acta Sci Vet*, 32(suppl. 1):223. (abstract).

Pincinato D. 2012. *Follicular dymanics and fertility in beef suckled cows synchronized with progesterone releasing devices and GnRH*. Cordoba, Argentina: Facultad de Ciencias Agropecuarias, Universidad Nacional de Cordoba. Thesis. 51 pp.

Pursley JR, Mee MO, Wiltbank MC. 1995. Synchronization of ovulation in dairy cows using PGF2α and GnRH. *Theriogenology*, 44:915-923.

Pursley JR, Fricke PM, Garverick HA, Kesler DJ, Ottobre JS, Stevenson JS, Wiltbank MC. 2001. NC-113 Regional Research Project. Improved fertility in noncycling lactating dairy cows treated with exogenous progesterone during Ovsynch. *In*: Midwest Branch ADSA 2001 Meeting, 2001, Des Moines, IA. Champaign, IL: ADSA. pp. 63. (abstract).

Sá Filho MF, Ayres H, Ferreira RM, Marques MO, Reis EL, Silva RC, Rodrigues CA, Madureira EH, Bó GA, Baruselli PS. 2010. Equine chorionic gonadotropin and gonadotropin-releasing hormone enhance fertility in a norgestomet-based, timed artificial insemination protocol in suckled Nelore (*Bos indicus*) cows. *Theriogenology*, 73:651-658.

Sá Filho OG, Meneghetti M, Peres RFG, Lamb GC, Vasconcelos JLM. 2009. Fixed-time artificial insemination with estradiol and progesterone for *Bos indicus* cows. II: strategies and factors affecting fertility. *Theriogenology*, 72:210-218.

Saldarriaga JP, Cooper DA, Cartmill JA, Stanko RL, Williams GL. 2005. Sincronización de la ovulación e inseminación artificial a tiempo fijo en ganado cruza *Bos indicu*s en Estados Unidos. *In*: VI Simposio Internacional de Reproducción Animal, 2005, Córdoba, Argentina. Córdoba: IRAC. pp 97-128.

Santos JEP, Narciso CD, Rivera F, Thatcher WW, Chebel RC. 2010. Effect of reducing the period of follicle dominance in a timed AI protocol on reproduction of dairy cows. *J Dairy Sci*, 93:2976-2988.

Small JA, Colazo MG, Kastelic JP, Mapletoft RJ. 2009. Effects of progesterone pre-synchronization and eCG on pregnancy rates to GnRH-based, timed-AI in beef cattle. *Theriogenology*, 71:698-706.

Souza AH, Ayres H, Ferreira RM, Wiltbank MC. 2008. A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. *Theriogenology*, 70:208-215.

Souza AH, Viechnieski S, Lima FA, Silva FF, Araújo R, Bó GA, Wiltbank MC, Baruselli PS. 2009. Effects of equine chorionic gonadotropin and type of ovulatory stimulus in a timed-AI protocol on reproductive responses in dairy cows. *Theriogenology*, 72:10-21.

Stevenson JS, Pursley JR, Garverick HA, Fricke PM, Kesler DJ, Ottobre JS, Wiltbank MC. 2006. Treatment of cycling and noncycling lactating dairy cows with progesterone during Ovsynch. *J Dairy Sci*, 89:2567-2578.

Veneranda G, Filippi L, Racca D, Romero G, Balla E, Cutaia L, Bó GA. 2006. Pregnancy rates in dairy cows treated with intravaginal progesterone devices and different fixed-time AI protocols. *Reprod Fertil Dev*, 18:118.

Veneranda G, Filippi L, Racca D, Cutaia L, Bó GA. 2008. Pregnancy rates in dairy cows treated with intravaginal progesterone devices and GnRH or estradiol benzoate and eCG. *Reprod Fertil Dev*, 20:91. (abstract).

Williams SW, Stanko RL, Amstalden M, Williams GL. 2002. Comparison of three approaches for synchronization of ovulation for timed artificial insemination in *Bos indicus*-influenced cattle managed on the Texas gulf coast. *J Anim Sci*, 80:1173-1178.

Wiltbank M, Lopez H, Sartori R, Sangsritavong S, Gumen A. 2006. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism.*Theriogenology*, 65:17-29.