Vol.57, n.3: pp. 361-366, May-June 2014
http://dx.doi.org/10.1590/S1516-89132014005000016
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

The Effects of Intramuscular or Intravenous Injections of Gonadotropin Releasing Hormone at Fixed-Time Artificial Insemination on Pregnancy Rates of Bos Indicus Beef Cows

Danilo Demeterco[1], Darren Dwayne Henry[2],Vitor Rodrigues Gomes Mercadante[2], Graham Cliff Lamb[2], Francisco Romano Gaievski[1], Bruno Weiss[1], Gustavo Nascimento Turbay[1], Márcio Saporski Segui[1], Romildo Romualdo Weiss[3], Melina Andreia Formighieri Bertol[3] and Luiz Ernandes Kozicki[1*]

[1]Escola de Ciências Agrárias e Medicina Veterinária; Pontificia Universidade Catolica do Paraná; São José dos Pinhais - PR - Brasil. [2]North Florida Research and Education Center; University of Florida; Marianna – Florida – USA. [3]Programa de Pós Graduação em Processos Biotecnológicos; Universidade Federal do Paraná; Curitiba – PR - Brasil

ABSTRACT

The effect of an intramuscular versus intravenous administration of gonadotropin releasing hormone (GnRH) at fixed-time AI (FTAI) on the pregnancy rates of crossbred Bos indicus *beef cows was evaluated. Pluriparous nursing calv cows (n=120) were synchronized as follows: d 0 cows received a 2.0 mg injection of estradiol benzoate (EB) and insertion of a controlled intravaginal progesterone releasing device containing 0.558 g of progesterone, d 8 removal of the progesterone device , a 0.15 mg injection of prostaglandin $F_{2\alpha}$ (PGF), a 1.0 mg injection of EB, and 400 IU injection of equine chorionic gonadotropin. Fifty-four hr after PGF, all cows were exposed to FTAI and a 0.084 mg injection of GnRH was administered either via Vena caudalis (n=60), or via Longissimus dorsi (n=60). Cows were inseminated with the same sire and by a single AI technician. Pregnancy was determined by the transrectal ultrasonography on d 40 after AI. Cows receiving the intravenous administration of GnRH had higher (P = 0.04) pregnancy rates than the cows receiving the intramuscular injection of GnRH (65 vs 46.6%, respectively). It was concluded that the intravenous administration of GnRH at the time of AI improved the pregnancy rates of crossbred* Bos indicus *beef cows submitted to FTAI.*

Key words: Gonadotropin releasing hormone, fixed-time artificial insemination, intravenous intramuscular administration

INTRODUCTION

Artificial insemination (AI) has quickly become one of the most effective tools to accelerate the genetic improvement of beef and dairy cattle herds. It allows the selection of desired characteristics through the use of proven bulls. The development of estrous synchronization protocols allows insemination of the without the need of heat detection, contributing to the widespread use of AI in beef cattle operations (Dobbins et al. 2009). The use of estrous synchronization protocols maximizes the use of time, labor and financial resources, and it increases the uniformity of the calf crop and shortens the breeding season (Dahlen et al. 2003). In the past 20 years, several synchronization protocols for FTAI have been developed. Pursley

*Author for correspondence: kozicki.l@pucpr.br

et al. (1998) developed the ovsynch protocol (administration of GnRH on day 0, followed prostaglandin $F_{2\alpha}$ day 7 and GnRH again on day 10 and FTAI 16 hours later) whereas Geary and Whittier (1998) established the Co-Synch protocol. The Co-Synch protocol calls for an injection of gonadotropin releasing hormone (GnRH) on d -10, prostaglandin F2α (PGF2α) on d -3 and GnRH with FTAI 48 to 72 h later.

An efficient hormonal protocol for FTAI must synchronize the follicular wave in order to obtain a ovalation in a predetermined time. GnRH administration is induced from the pituitary the pre-ovulatory peak of LH within 2 to 4 h and ovulation within 24 to 36 h (Smith et al. 2012), depending on the developmental stage of the follicle (Geary et al. 2000; Atkins et al. 2008). Today, most protocols for FTAI include the use of intravaginal implants of progesterone (P4), PGF2α (IM) and GnRH (IM), which may achieve the pregnancy rates similar to conventional AI (Viana et al. 2008), without observation of estrus, providing less labor and animal stress (Geary and Whittier 1998; Lamb et al. 2001, 2006; Walker et al. 2005b). The use of the eCG in cows on d 8 after the follicular wave has been synchronized with EB, results in a larger and more mature follicle. This improved the synchronization of ovulation, increasing the pregancy rate at FTAI helped by the GnRH administration (Sá Filho et al. 2010).

The pregnancy rate with FTAI can decrease substantially if follicles, which have not yet reached the ideal size, are induced to ovulation by GnRH. However, this can be avoided if eCG is administered at the time of P4 removal (Small et al. 2009). Injections of eCG and PGF2α on the day of P4 removal promotes greater growth of the preovulatory follicle and ovulation, mainly in the cows with lesser BCS. Protocols utilizing P4 intravaginally, GnRH and PGF2α, induce estrus in a large percentage of animals between 48 and 60 h after P4 implant removal (Schmitt et al. 1996; Martinez et al. 2002), improving the fertility of animals synchronized. The application of GnRH with FTAI may be an option to increase the fertility rates in beef herds (Walker et al. 2005a). Routinely, GnRH has been used intramuscularly to induce the ovulation when working with FTAI (Smith et al. 2012). Estrous synchronization protocols for FTAI, involving GnRH at 48 h after the application of PGF2α, induce LH preovulatory pulses, which assist the ovulation of the dominant follicle (DF) and define the best time for AI (Fernandes et al. 2001; Dahlen et al. 2003). Using FTAI, higher pregnancy rates (57.1%) were observed when administered the GnRH at 54 h after the removal of P4 plus PGF2α when compared with the same protocol without the application of GnRH (48.7%) (Twagiramungu et al. 1995). Dahlen et al. (2003) reported that the animals submitted to CO-Synch protocol without GnRH demonstrated pregnancy rates 17% lower than the animals treated with CO-Synch protocol + GnRH, concluding that the additional cost of hormone would be compensated by higher pregnancy rate. Barros et al.(2000) reported that the administration of GnRH (intramuscular) accelerated the ovulation by about 30 h, resulting in ovulation within 12 h of GnRH administration. The use of GnRH on the day of FTAI after previous use of P4 implant and eCG (IM) increases the pregnancy rates in the pluriparous cows because eCG assists the growth of larger follicles and GnRH synchronizes the ovulation (Drost and Thatcher 1992; Peters 2005; Small et al. 2009). GnRH administered intramuscularly can induce the pulses of LH after 1 h in 75% of animals to better synchronize the ovulation of developed follicles (McLeod et al. 1991; Yavas and Walton 2000).

Routinely, GnRH administration in the CO-Synch protocol has been given intramuscularally (Small et al. 2009; Sá Filho et al. 2010). There are few studies on the use of GnRH intravenously in cattle. Lapwood and Wilson (1978) reported studies of GnRH injected intravenously in sheep, resulting in rapid rise in LH concentration in the blood plasma, with the first peak before 90 min. Homeida et al. (1991) reported the intravenous administration of 50 mg of GnRH in camels injected every 2 h, resulting in 75% of estrus in the treated animals with significant increase in LH concentrations and ovulation. Grasseli et al. (1993) administered continuous intraveniously GnRH in prepubertal heifers, resulting in rapid release of LH preovulatory peaks after 2-4 h. Bas et al. (2012) administered GnRH via intramuscular and intrauterine observing no differences in the ovulation rate between the two groups of dairy cows. This study hypothesized that the intravenous administration of GnRH would result in larger and faster peaks of GnRH than the intramuscular application, not only because the absortion would be better and faster, but also concentration in the target organ would be higher. Based on this hypothesis, the aim of this study was to evaluate the pregnancy rates in the crossbred beef cattle (Nellore x Tabapuã) submitted to the P4 protocol for 8 days, PGF2 alpha and eCG, plus GnRH intramuscular or intravenous administration at FTAI.

MATERIALS AND METHODS

The study was conducted in the central region of Parana state (Parallel 24°48.328' S, 052°03.311' W; altitude 854 meters). A total of 120 cows nursing calf with and crossbred cows (Nellore x Tabapuã), average age of 5 years±1.3, BCS 2.7 (1=thin; 5=obese; Edmonson et al. 1989) were measured in April, outside of the reproductive season. The animals were kept on pasture (*Brachiaria decubens* and *Brachiaria brizantha*) and mineral and water were supplied *ad libitum*. Cows were randomly divided into two groups (G1 and G2) of 60 animals each and submitted to hormonal protocols (P4 + BE at d 0 and P4 removal + PG + eCG + BE at d 8) and GnRH + FTAI 54 h after (Fig. 1). The difference between the groups was the site of administation, intramuscular or intravenous, of GnRH. Group 1 received on the day of FTAI a dose of 0.084 mg of buserelin acetate (GnRH) intravenously (*Vena caudalis*) and G2 the same dose of GnRH intramuscularly in the *Longissimus dorsi* muscle. Forty days after the FTAI, pregnancy diagnosis using ultrasonography (Aloka SSD-500v Ultrasound ®, 5-Mhz, Japan) was performed.

Protocols

The animals of both the groups received an intravaginal device containing progesterone (0.558 g, Cronipress ® Monodose, Biogenesis Bagó, Curitiba-PR) and an injection of estradiol benzoate (estradiol benzoate, 2.0 mg Bioestrogen ®, intramuscular) on day zero. On d 8, P4 was removed and animals received a dose of PGF2alpha (d-Cloprostenol, 0.15 mg, Croniben ®, intramuscular), a dose of estradiol benzoate (1.0 mg, intramuscular) and a dose of 400 IU of eCG (Novormon ®, Intervet, Sao Paulo) intramuscularly. Fifty-four hours later, G1 received an injection of GnRH (Buserelin acetate, 0.084 mg, Sincroforte ®, intravenously) and G2 recieved the same dose (intramuscularly), before the FTAI (Fig. 1).

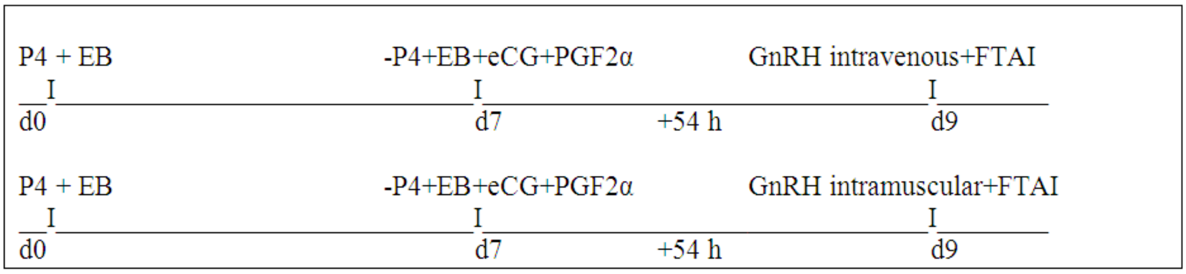

Figure 1 - Hormone protocols administered on 120 crossbred cows to FTAI.

Progesterone (0.558 g, Cronipress ® Monodose, Biogenesis Bagó, Curitiba-PR); estradiol benzoate (estradiol benzoate, 2 mg Bioestrogen ®, intramuscular); PGF2α (d-Cloprostenol, 0.15 mg, Croniben ®, intramuscular); 400 IU of eCG (Novormon ®, intervet, Sao Paulo); GnRH (buserelin acetate, 0.084 mg, Sincroforte ®)

AI was performed by an artificial insemination certified technician, using frozen semen from the same bull (Nelore Mocho). The semen was from the Central Artificial Insemination, packed in 0.25 ml straws and stored in liquid nitrogen canisters. The thawing of the semen was performed in a water bath type electronic semen defroster (Cryofarm ®, Italy) for 30 seconds at a temperature of 37˚C. The semen deposition was in the uterine body, following the instructions of the Manual for Andrology and Evaluation of Animal Semen (Manual for Andrology and Animal Semen Evaluation, 1998).

Statistical analysis

Data were analyzed using the procedure FREQ of SAS (SAS Inc, Cary, NC, 1996). The level of significancy was 5% ($p<0.05$).

RESULTS AND DISCUSSION

As displayed on Table 1, there was a significant difference in the pregnancy rate, which favored the group that received intravenous GnRH (65.0% versus 46.6%) of the intramuscular group (P = 0.04).

Table 1 - Pregnancy Rate in Crossbred cows Nellore x Tabapuã submitted to fixed-time artificial insemination using GnRH intramuscularly or intravenously on day of AI.(n=120).

Groups	Pregnancy[1]	
	N	%
G1 (GnRH - intravenous)	39 / 60	65,0[a]
G2 (GnRH - intramuscular)	28 / 60	46,6[b]

[1]Different letters in the same column indicate significance at *P*<0.05.

The Co-synch protocol has earned better acceptance among the users when an intravaginal progesterone device was added, making it a good alternative for the synchronization of estrus in beef cows (Lamb et al. 2012). Busch et al. (2008) tested the protocol CO-Synch + CIDR in beef cattle, getting 61% pregnancy rate applying the GnRH + FTAI at 54 h after P4 implant removal. In the present study, the animals were submitted to a protocol GnRH + FTAI applied at 54 h after P4 removal, varying however, the form of adminstration. Traditionally, the GnRH is administered intramuscularly (Atkins et al. 2008). But in this study, one group of animals received GnRH intravenously. In this case, the hypothesis was that G1 (intravenous GnRH) could improve the pregnancy rate when compared and confirmed with G2, based on the difference of pregnancy rate (65.0% versus 46.6% respectively) ($P<0.04$) between the groups.

Literature about the application of GnRH intramuscularly for the purpose of inducing ovulation is plentiful (Pursley et al. 1998; Geary and Whittier 1998; Barros et al. 2000; Dobbins et al. 2006; Busch et al. 2008), but data supporting the intravenous administration, especially in the bovine is lacking (Graselli et al. 1993). Lapwood and Wilson (1978) administered intravenous GnRH in sheep observing rapid and marked LH elevation in the plasma within 15 to 20 min. Camels received GnRH intravenously had 75% of ovulation (Homeida et al. 1991). Due to the scarcity of the studies about intravenous GnRH, it became difficult to compare the present findings and those of other researchers.

The pregnancy rate of G1 in this study was higher than that obtained by Busch et al. (2008) (61.0%) when applying GnRH (IM) + FTAI at 54 h after the withdrawal of P4. The difference between the groups of cows in this study that received the GnRH intravenous and data from Busch et al. (2008) (intramuscular GnRH), was more related to the method of application of GnRH on the day of FTAI and the application of ECG on d7, along with intravenous GnRH, positively influenced the results. The intravenous GnRH would be more efficient to induce faster and more punctual effects on the ovulatory follicle (OF), improving the rate of pregnancy. This became clear in this study comparing the two groups. The hypothesis was that the intravenous GnRH acted more efficiently on the OF, decreasing the time of interval between the application of GnRH and ovulation, accelerating the final maturation of the OF, providing an "optimum time" of fertilization of gametes in oviducts, reinforcing reports of Dalton and Saacke (2007) that AI should be performed at to the ovulation time, but not too late so that the oocyte becomes too old. Barros et al. (2000), in a study of Nellore cows, reported that the administration of GnRH (intramuscular) 24 h after the application of prostaglandin F 2 alpha (day of P4 removal) synchronized the ovulation within 12 h when compared with the animals that had not received GnRH. Smith et al. (2012) reported that from 2 to 4 h after the application, the GnRH induced the release of ovulatory surge of LH and ovulation occurred within 25 to 30 h (after the release of LH). As the oocyte has a viability from 8 to 10 h in the oviduct and sperm remain viable for 18 to 20 h (Smith et al. 2012), the administration of GnRH will most efficientty assist the synchronization of ovulation. In this case, the use of intravenous GnRH might have provided an improved synchronization of ovulation, increasing the pregnancy rate at FTAI corroborating the report of Sá Filho et al. (2010).

Another relevant factor in the study was that this was conducted during the out of breeding season (April), when the grasses were poorer in nutritional content and the rainfalls were scarce. In addition, the cows in this experiment were calved in January. To avoid calving this late, they remained open until the next breeding season (October-November) and all were subjected to the protocol for FTAI. This showed good results considering the pregnancy rate, because the cows already had some difficulties to become pregnant in the previous breeding season. Also in this case, the intravenous administration of GnRH might have better influenced, releasing the pre-ovulatory peak of LH on the synchronization of the ovulation (Smith et al. 2012) in a larger number of animals than on the intramuscular group. It was concluded that the intravenous administration of GnRH increased the pregnancy rate of the animals at FTAI when compared with the cows that received intramuscular GnRH and also saved the time of animal management.

REFERENCES

Atkins JA, Busch JF, Bader DF, Schafer DJ, Lucy MC, Patterson DJ, Smith MF. GnRH-induced ovulation in heifers: Effects of stage of follicular wave. *J Anim Sci.* 2008; 86(1):83-93.

Barros CM, Moreira MBP, Figueiredo RA, Teixeira AB, Trinca LA. Synchronization of ovulation in beef cows (Bos indicus) using GnRH, $PGF_{2\alpha}$ and estradiol benzoate. *Theriogenology*. 2000; 53:1121-1134.

Bas S, Pinto CG, Day ML, Schuenemann GM. Effect of intrauterine administration of gonadotropin relasing hormone on serum LH concentrations in lactating dairy cows. *Theriogenology*. 2012;78:1390-1397.

Busch DC, Schafer DJ, Wilson DJ, Mallory DA, Leitman NR, Haden JK, et al. Timing of artificial insemination in postpartum beef cows following administration of the CO-Synch + controlled internal drug-release protocol. *J Anim Sci*. 2008;86:1519-1525.

Dahlen CR, Lamb GC, Zehnder CM, Miller LR, Dicostanzo A. Fixed-time insemination in peripuberal, lightweight replacement beef heifers after estrus synchronization with $PGF_{2\alpha}$ and GnRH. *Theriogenology*. 2003;59:1827-1837.

Dalton JC, Saacke RG. Parâmetros da qualidade do sêmen para programas de sincronização. Novos Enfoques na Produção e Reprodução de Bovinos. 2007;11:154-161.

Dobbins CA, Eborn DR, Tenhouse DE, Breiner RM, Johnson SK, Marston TT, et al. Insemination timing affects pregnancy rates in beef cows treated with CO-Synch protocol including an intravaginal progesterone insert. *Theriogenology*. 2009;72:1009-1016.

Dobbins CA, Tenhouse DE, Eborn DR, Harmony KR, Johnson SK, Stevenson JS. Conception rates after altered timing of AI associated with the CO-Synch + CIDR protocol. *J Anim Sci*. 2006;84:50 (Abstr.)

Drost M, Thatcher WW. Application of gonadotrophin releasing hormone as therapeutic agent in animal reproduction. *Anim Reprod Sci*. 1992;28:11-19.

Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster GA. body condition scoring chart for Holstein dairy cows. *J Dairy Sci*. 1989;72:39-41.

Fernandes P, Teixeira AB, Crocci AJ, Barros CM. Timed artificial insemination in beef cattle using GnRH agonist, $PGF_{2\alpha}$ and estradiol benzoate. *Theriogenology*. 2001;55:1521-1532.

Geary TW, Downing ER, Bruemmer JE, Whittier JC. Ovarian an destrus response of suckled beef cows to the select synch estrus sybchronization protocol. *Profes Anim Scient.* 2000; 16:1-5.

Geary TW, Whittier JC. Effects of a timed insemination following synchroonization of ovulation using the Ovsynch or CO-Synch protocol in beef cows. *Profes Anim Scient*. 1998; 14:217-220.

Grasselli F, Baratta M, Tamanini C. Effects of a GnRH analogue (buserelin) infused via osmotic minipumps on pituitary and ovarian activity of prepubertal heifers. *Anim Reprod Sci*.1993;32:153-161.

Homeida AM, Taha AAM, Khalil MGR, Hoppen HO. Secretion of LH and progesterone after intravenous administration of GnRH in the camel (Camelus dromedarius). *Anim Reprod Sci*.1991;25:67-72.

Lamb GC, Larson JE, Geary TW, Stevenson JS, Johnson SK, Day ML, et al. Sinchronization of estrus and artificial insemination in replacement beef heifers using gonadotropin-releasing hormone, prostaglandin $F_{2\alpha}$ and progesterone. *J Anim Sci*. 2006; 84:3000-3009.

Lamb GC, Stevenson JS, Kesler DJ, Garverick HA, Brown DR, Salfen BE. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin $F_{2\alpha}$ for ovulation control in postpartum suckled beef cows. *J Anim Sci*. 2001;79:2253-2259.

Lamb GC. Control of estrus in cows, *Proceedings of the Applied Reproductive Strategies in beef cattle*. Sioux Falls, South Dakota, 2012, :85-97.

Lapwood KR, Wilson PR. Pituitary and gonadal secretory responses of rams following intravenous infusion or injection of graded doses of GnRH. *Theriogenology*. 1978; 9:417-428.

Manual Para Exame Andrológico E Avaliação De Sêmen Animal. 2.ed. Belo Horizonte: Colegio Brasileiro de Reprodução Animal. 1998. 49p

Martínez MF, Kastelic JP, Adams GP, Cook B, Olson WO, Mapletoft RJ. The use of progestins in regiments for fixed-time artificial insemination in beef cattle. *Theriogenology*. 2002;57:1049-1049 (Abstr.).

Mcleod BJ, Dodson SE, Peters AR, Lamming GE. Effects of a GnRH agonist (buserelin) on LH secretion in post-partum beef cows. *Anim Reprod Sci*. 1991; 24:1-11.

Peters AR. Veterinary clinical application of GnRH – questions of efficacy. *Anim Reprod Sci*. 2005;88:155-167.

Pursley JR, Silcox RW, Wiltbank MC. Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. *J Anim Sci*. 1998; 81:2139-2144.

Sá Filho MF, Baldrighi JM, Sales JNS, Crepaldi GA, Carvalho JBP, Bó GA, et al. Equine chorionic gonadotropin and gonadotropin-releasing hormone enhance fertility in a norgestomet-based, timed artificial insemination protocol in sucklet nelore (Bos indicus) cows. *Theriogenology*. 2010;73:651-658.

Schmitt EJP, Drost M, Diaz T, Roomes C, Thatcher WW. Effect of a gonadotropin releasing hormone agonist on follicle recruitment and pregnancy rate in cattle. *J Anim Sci*. 1996;74:154-161.

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

Smith MF, Pohler KG, Perry GA, Patterson D. Physiological factors that affect pregnancy rate to artificial insemination in beef cattle. *Proceedings Applied Reproductive Strategies in beef cattle*, December 3-4, 2012, Sioux falls, South Dakota, p. 33-51.

Twagiramungu H, Roy GL, Laverdiére G, Dufour JJ. Fixed-time insemination in cattle after synchronization of estrus and ovulation with GnRH and prostaglandin. *Theriogenology*. 1995;43:341 (Abstr.)

Viana GNO, Kozicki LE, Weiss RR, Segui MS, Meirelles C, Efing AC, et al. Comparação de diferentes protocolos para a sincronização de estro e inseminação artificial em tempo fixo em vacas da raça nelore em anestro pós-parto. *Arch Vet Sci*. 2008;13:247-254.

Walker RS, Burns PD, Whittier JC, Sides GE, Zalesky DD. Evaluation of gonadotropin-releasing hormone and insemination time using CO-Synch protocol in beef cows. *Profes Anim Scient*. 2005b; 21:190-194.

Walker RS, Enns RM, Geary T W, Mortimer RG, Lashell BA, Zalesky DD. Evaluation of gonadotropin-releasing hormone at fixed-time artificial insemination in beef heifers synchronized using a modified CO-Synch plus controlled internal device release protocol. *Profes Anim Scient*. 2005a; 21:449-454.

Yavas Y, Walton JS, Induction of ovulation in portpartum suckled beef cows: A review. *Theriogenology*. 2000;54:1-23.

Received: May 02, 2013;
Accepted: January 16, 2014.