Original Article
J Vet Sci 2015, 16(3), 367-371 · http://dx.doi.org/10.4142/jvs.2015.16.3.367

Influence of category—heifers, primiparous and multiparous lactating cows—in a large-scale resynchronization fixed-time artificial insemination program

Márcio de Oliveira Marques[1], Fábio Morotti[2], Camila Bizarro da Silva[2], Mario Ribeiro Júnior[1], Rubens César Pinto da Silva[1], Pietro Sampaio Baruselli[3], Marcelo Marcondes Seneda[2,*]

[1]*Geraembryo, Cornélio Procópio 86300-000, Brazil*
[2]*Laboratory of Animal Reproduction, Department of Veterinary Clinics, Agricultural Science Center, State University of Londrina, Londrina 86051-990, Brazil*
[3]*Department of Animal Reproduction, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo 05508270, Brazil*

This study was conducted to evaluate the influence of category (heifers, primiparous or multiparous cows) on pregnancy rates in a large scale resynchronization ovulation program. Nelore heifers (n = 903), primiparous lactating cows (n = 338) and multiparous lactating cows (n = 1,223) were synchronized using a conventional protocol of estradiol/P4-based fixed-time artificial insemination (FTAI). Thirty days after ultrasonography, females who failed the first FTAI were resynchronized with the same hormonal protocol prior to a second FTAI. The pregnancy status of each cohort was evaluated by ultrasonography 30 days after each FTAI. The average conception rate after the first FTAI and resynchronization was 80.5%. Heifers had a higher conception rate (85%) than primiparous (76%) or multiparous cows (78%; $p = 0.0001$). The conception rate after the first FTAI was similar among heifers (57%), primiparous cows (51%) and multiparous cows (56%; $p = 0.193$). After the second FTAI, heifers exhibited a higher conception rate (66%) than primiparous or multiparous cows (51%; $p = 0.0001$). These results demonstrate the feasibility of resynchronization in large beef herds for providing consistent pregnancy rates in a short period of time. We also demonstrated that ovulation resynchronization 30 days after FTAI is particularly effective for heifers, providing a conception rate of up to 66%.

Keywords: *Bos indicus*, conception rate, fixed-time artificial insemination, hormonal treatment, resynchronization

Introduction

Reproductive efficiency is an important strategy for maximizing the productivity of cattle herds. Among the current strategies used to improve reproductive performance, ovulation synchronization has become the best technique for optimizing herd management and providing genetic improvements with greater efficiency.

Among beef cattle, approximately 40 to 60% of inseminated females become pregnant after the first cycle of fixed-time artificial insemination (FTAI), with slight variations in rates depending on several factors including the animals, farm management, body condition score, postpartum time and hormonal treatments [2,3,11]. Therefore, females that do not conceive after the first service need to be re-inseminated as soon as possible to increase the reproductive performance of the herd [4,6,7,19]. If these females are not re-inseminated in a short period of time, the interval between calving and conception increases, decreasing the reproductive efficiency of the herd.

The resynchronization of females who failed the first FTAI is an effective strategy to increase the number of pregnancies achieved by AI during a short breeding season [19]. Ovulation resynchronization can increase the percentage of non-pregnant cows that are subjected to a second insemination [13] and be an advantageous strategy when the number of bulls is insufficient to mate with all of the cows that did not become pregnant after the first FTAI [8]. During resynchronization, all cows inseminated in the first round of FTAI should be evaluated by ultrasonography, and those that did not become pregnant receive the same hormonal protocol for a second round of FTAI [7,18].

One of the most common hormonal protocols for FTAI or resynchronization in cattle involves the use of a progesterone

Received 28 Aug. 2014, Revised 18 Feb. 2015, Accepted 7 Mar. 2015
*Corresponding author: Tel: +55-43-3371-5622; Fax: +55-43-3371-4063; E-mail: mseneda@uel.br
pISSN 1229-845X
eISSN 1976-555X

source, such as an intravaginal device or norgestomet ear implant, in combination with 1 mg of estradiol benzoate (EB), 0.5 mg of estradiol cypionate (EC) [10,19] or 100 μg of GnRH [16]. Temporary calf removal (TCR) for 48 to 54 h or 300 IU of equine chorionic gonadotropin (eCG) have also been successfully applied at the time of device withdrawal and in conjunction with a luteolytic agent and inducer of ovulation to improve the reproductive performance of resynchronized *Bos indicus* cows [4].

Resynchronization protocols have been evaluated in different categories of female beef and dairy cattle (*Bos taurus*) [19], non-lactating cows and beef heifers (*Bos taurus*) [7,8], multiparous lactating beef cows (*Bos indicus*) [4], non-lactating cows and cycling beef heifers (*Bos indicus*) and crossbred cows from *Bos indicus* and *Bos taurus* parents [16]. Nevertheless, no studies have examined different cohorts in a single resynchronization study, particularly with heifers or newly calved cows. Therefore, the present study was conducted to investigate the conception rate in heifers, primiparous and multiparous lactating cows after a large-scale FTAI program, followed by resynchronization using an estradiol/P4-based protocol.

Materials and Methods

Location, animals and management

This study was conducted during the 2011/2012 breeding season in South America at latitude 21°15'23" and longitude 52°2'29". The climate in this region is tropical, with an average temperature of 24°C and a rainy season from November to January. Data for this study were collected from *Bos indicus* (Nelore) herds including heifers, as well as primiparous and multiparas lactating cows (n = 2,464) that were selected to receive a conventional protocol of FTAI in two commercial beef farms in Southern Brazil under the same management.

Heifers (24 to 27 months old) and cows (36 to 180 months old) were selected based on an adequate body condition score (BCS) and postpartum period, normal estrous cycles and health status. The primiparous and multiparous lactating cows were 30 to 50 days postpartum (average 45 days), and only females with a BCS between 2.0 and 4.0 (average 3) on a scale of 1 to 5 [1] were used for the FTAI program. The herds were kept in an extensive system that allowed for continuous grazing of *Brachiaria spp*. and were given *ad libitum* access to mineralized salt and water.

Hormonal treatment

On a random day of the estrous cycle (Day 0), heifers (n = 903), primiparous lactating cows (n = 338) and multiparous lactating cows (n = 1,223) underwent a conventional estradiol/P4-based FTAI protocol. The protocol consisted of the insertion of an intravaginal device containing 1 g of progesterone (P4) (DIB; MSD Animal Health, Brazil; cows) or an ear implant containing 3 mg of norgestomet (Crestar; MSD Animal Health; heifers) and intramuscular (i.m.) administration of 2 mg of EB (Gonadiol; MSD Animal Health). On Day 8, the devices were removed and the animals received i.m. injections containing 250 μg of cloprostenol (Ciosin; MSD Animal Health), 300 IU of eCG (Novormon; MSD Animal Health) and EC (1.0 mg for lactating cows and 0.5 mg for heifers, ECP; Zoetis, Brazil). All females were inseminated by two experienced inseminators using frozen-thawed semen of bulls 48 h after device removal (Fig. 1).

Thirty days after the first FTAI (Day 28 to 32), all animals were subjected to a pregnancy diagnosis by transrectal ultrasonography (Aloka SSD 500; Aloka, Japan) and non-pregnant females received the same hormonal treatment described above.

Heifers received norgestomet due to poor estrus synchronization results when a new P4-releasing intravaginal device (first use) was used [5,9,14,17]. To resynchronize ovulation, all females were implanted with a previously used P4 source (device or implant) for 8 days. After their initial use, the devices/implants were individually washed with water, then soaked in a solution of ascorbic acid, citric acid and lactic acid (Kilol-L; Quinabra, Brazil) for approximately 10 minutes. Thereafter, the devices were dried and stored at room temperature until use.

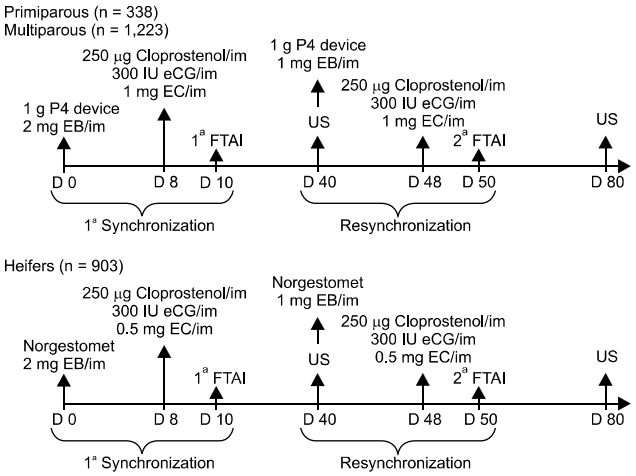

Fig. 1. Design of hormonal treatments used for fixed-time artificial insemination (FTAI) and estrus resynchronization in Nelore heifers, primiparous and multiparous lactating cows. On Day 0, all animals received a P4 source (intravaginal device for the cows; ear implants for the heifers) in combination with 2 mg of estradiol benzoate (EB). Eight days later, the devices/implants were removed, and all females received 250 μg of cloprostenol, 300 IU of eCG, and 1 mg (cows) or 0.5 mg (heifers) of EC. FTAI was performed 48 h after the device/implant was removed. Thirty days after the first FTAI (28 to 32 days), all females were assessed by ultrasonography, and the non-pregnant bovines were resynchronized with the same hormonal treatment. The pregnancy rate was assessed by ultrasound 30 days after FTAI.

Pregnancy diagnosis

Pregnancy diagnosis was performed by ultrasonography 30 days after each FTAI cycle. The first evaluation occurred on Day 30 (28 to 32 days), while the second was conducted 30 days later. In both evaluations, females were scanned by transrectal ultrasonography using an Aloka SSD-500 ultrasound equipped with a 5 MHz linear transducer (Aloka). The conception rate was calculated by dividing the number of pregnant cows by the total number of females inseminated on Day 30 and 30 days after the second FTAI.

Statistical analysis

The data are presented as proportions. The conception rates corresponding to the first FTAI, second FTAI and overall rate within 30 days of the second FTAI (first FTAI + resynchronization) were compared across categories (heifers, primiparous cows and multiparous cows).

Statistical analysis was performed using Minitab. The significance level for rejecting the null hypothesis was $p \leq 0.05$. A chi-square test was used to determine differences in conception rates among the categories of females.

Results

Of the 2,464 female bovines subjected to an estradiol/P4-based FTAI protocol followed by ovulation resynchronization, 1,985 animals became pregnant, resulting in an average conception rate of 81% 30 days after the second FTAI (Table 1). Heifers exhibited a higher conception rate (85%; 770/903) than the primiparous cows (76%; 257/338) or multiparous cows (78%; 958/1,223; $p = 0.0001$).

The average conception rate after the first FTAI was 55% (1,367/2,464), and there were no differences between the conception rates of the heifers (57%; 514/903), primiparous cows (51%; 173/338) or multiparous cows (56%; 680/1,223; $p = 0.193$). The average conception rate after the second FTAI (resynchronization) was 56% (618/1,097), and the conception rate was higher in heifers (66%; 256/389) than in the primiparous cows (51%; 84/165) and multiparous cows (51%; 278/543; $p = 0.0001$).

Discussion

Data from the current study demonstrated that it is possible to achieve high pregnancy rates in a short period of the breeding season using only FTAI after resynchronization of heifers, primiparous and multiparous lactating cows. The higher pregnancy rate after the second FTAI in heifers should be considered strategic information for large scale AI programs in beef herds.

Thirty days after the first FTAI procedure, we used 1 mg of EB in combination with a P4 source (intravaginal device or norgestomet ear implant) to induce a new follicular wave. A recent study evaluated the use of resynchronization employing a progestin-based FTAI protocol in beef cattle (*Bos indicus*) with two different inducers of new follicular waves (EB *vs*. GnRH). This study showed that both inducers resulted in similar pregnancy rates (70.5% and 63.2%, respectively). Additionally, 1 mg of EB 22 days after FTAI did not affect pre-established pregnancy [16]. Another study of previously inseminated lactating beef cows (*Bos taurus*) used resynchronization with P4 + 1 mg of EB or P4 + 0.5 mg of EC. The P4 sources in combination with estrogen treatment did not decrease the conception rates, resulting in pregnancy rates of 86% and 65%, respectively [19].

In the present study, the conception rate following the first FTAI protocol did not differ among the cohorts evaluated (heifers (57%), primiparous cows (51%) and multiparous cows (56%)). Additionally, these results were very similar to those observed by Campos *et al*. [4], who achieved conception rates ranging from 47 to 54% in Nelore multiparous cows after evaluating data from the first FTAI in an estrus resynchronization program.

The second FTAI resulted in higher conception rates in heifers than cows (66% *vs*. 51%, respectively), demonstrating that the category influences the conception rate of re-inseminated females. Some field studies have shown that *Bos indicus* cows exhibit different pregnancy rates following the first and second FTAI depending on the category of the resynchronized females

Table 1. Conception rates of Nelore heifers, primiparous and multiparous lactating cows after the first FTAI and resynchronization of females who failed the first FTAI

Categories	Conception rate (1[a] FTAI)	Conception rate (2[a] FTAI)	Conception rate (1[a] FTAI + 2[a] FTAI)
Heifers	57%[a] (514/903)	66%[a] (256/389)	85%[a] (770/903)
Primiparous	51%[a] (173/338)	51%[b] (84/165)	76%[b] (257/338)
Multiparous	56%[a] (680/1,223)	51%[b] (278/543)	78%[b] (958/1,223)
Mean or total	55% (1,367/2,464)	56% (618/1,097)	81% (1,985/2,464)
p value	0.193	0.0001	0.0001

Rates followed by different superscript letters (a or b) within the same column were significantly different ($p \leq 0.05$). FTAI: fixed-time artificial insemination.

[4,16]. In our field experience, there is a decrease in pregnancy rate following the second FTAI relative to the rate after the first FTAI of primiparous cows. In the present study the pregnancy rate was similar when a second synchronization of ovulation was performed.

The resynchronized females from the present study achieved an average pregnancy rate of approximately 80% (after both the first and second FTAI) in only 80 days of the breeding season. Despite the better efficiency of resynchronization in heifers (85%), this protocol also provided promising results in primiparous (76%) and multiparous (78%) cows. By resynchronizing cycling heifers and non-lactating cows (*Bos indicus* and crossbred ½ *Bos indicus* ½ *Bos taurus*), Sá Filho *et al*. [16] achieved a 75% pregnancy rate, which was similar to the data described here. Similarly, Campos *et al*. [4] reported pregnancy rates of 76.6% and 74% following the resynchronization of lactating cows treated with 300 UI of eCG or temporary calf removal, respectively.

Lactating cows in the postpartum period represent a category of females with higher energy requirements [3]. During this period, the cow's nutritional status and presence of the calf exert strong influences on the female's energetic balance that could negatively influence breeding potential and the success of FTAI [12,15]. This is particularly true in primiparous cows, which have the highest energy demands [20]. Remarkably, in the present study, primiparous cows achieved a conception rate similar to multiparous cows that was similar to the average found in other studies of multiparous cows (74–76%) [4].

In conclusion, ovulation resynchronization of females who failed the first FTAI is a feasible and effective technique, providing an average conception rate of 80% in beef cattle. The best result was found in heifers, with a 66% pregnancy rate being observed after the second FTAI and an 85% rate after the two FTAI procedures.

Acknowledgments

The authors thank Geraembyo and the Brazilian National Council for Scientific and Technological Development (CNPq).

Conflict of Interest

There is no conflict of interest.

References

1. **Ayres H, Martins CM, Ferreira RM, Mello JE, Dominguez JH, Souza AH, Valentin R, Santos ICC, Baruselli PS.** Effect of timing of estradiol benzoate administration upon synchronization of ovulation in suckling Nelore cows (*Bos indicus*) treated with a progesterone-releasing intravaginal device. Anim Reprod Sci 2008, **109**, 77-87.
2. **Baruselli PS, Marques MO, Carvalho NAT, Madureira EH, Campos Filho EP.** Effect of different treatments for timed artificial insemination on the reproductive efficiency in lactating beef cows. Rev Bras Reprod Anim 2002, **26**, 218-221.
3. **Baruselli PS, Reis EL, Marques MO, Nasser LF, Bó GA.** The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. Anim Reprod Sci 2004, **82-83**, 479-486.
4. **Campos JT, Marinho LSR, Lunardelli PA, Morotti F, Seneda MM.** Resynchronization of estrous cycle with eCG and temporary calf removal in lactating *Bos indicus* cows. Theriogenology 2013, **80**, 619-623.
5. **Carvalho JBP, Carvalho NAT, Reis EL, Nichi M, Souza AH, Baruselli PS.** Effect of early luteolysis in progesterone-based timed AI protocols in *Bos indicus*, *Bos indicus* × *Bos taurus* and *Bos taurus* heifers. Theriogenology 2008, **69**, 167-175.
6. **Cavalieri J, Smart VM, Hepworth G, Ryan M, Macmillan KL.** Ovarian follicular development and hormone concentrations in inseminated dairy cows with resynchronized estrous cycles. Theriogenology 2008, **70**, 946-955.
7. **Colazo MG, Kastelic JP, Mainar-Jaime RC, Gavaga QA, Whittaker PR, Small JA, Martinez MF, Wilde RE, Veira DM, Mapletoft RJ.** Resynchronization of previously timed-inseminated beef heifers with progestins. Theriogenology 2006, **65**, 557-572.
8. **Colazo MG, Kastelic JP, Small JA, Wilde RE, Ward DR, Mapletoft RJ.** Resynchronization of estrus in beef cattle: ovarian function, estrus and fertility following progestin treatment and treatments to synchronize ovarian follicular development and estrus. Can Vet J 2007, **48**, 49-56.
9. **Dias CC, Wechsler FS, Day ML, Vasconcelos JLM.** Progesterone concentrations, exogenous equine chorionic gonadotropin, and timing of prostaglandin $F_2\alpha$ treatment affect fertility in postpuberal Nelore heifers. Theriogenology 2009, **72**, 378-385.
10. **El-Zarkouny SZ, Stevenson JS.** Resynchronizing estrus with progesterone or progesterone plus estrogen in cows of unknown pregnancy status. J Dairy Sci 2004, **87**, 3306-3321.
11. **Lamb GC, Dahlen CR, Larson JE, Marquezini G, Stevenson JS.** Control of the estrous cycle to improve fertility for fixed-time artificial insemination in beef cattle: a review. J Anim Sci 2010, **88** (Suppl E), E181-192.
12. **Marquezini GHL, Mercadante VRG, Olson KC, Jaeger JR, Perry GA, Stevenson JS, Lamb GC.** Effects of equine chorionic gonadotropin on follicle development and pregnancy rates in suckled beef cows with or without calf removal. J Anim Sci 2013, **91**,1216-1224.
13. **McDougall S, Loeffler SH.** Resynchrony of postpartum dairy cows previously treated for anestrus. Theriogenology 2004, **61**, 239-253.
14. **Meneghetti M, Sá Filho OG, Peres RFG, Lamb GC, Vasconcelos JLM.** Fixed-time artificial insemination with estradiol and progesterone for *Bos indicus* cows I: basis for development of protocols. Theriogenology 2009, **72**, 179-189.
15. **Perea FP, De Ondiz AD, Palomares RA, Hernández HJ, González R, Soto ER.** Control of postpartum anestrous with an intra-vaginal progesterone device plus eCG or calf

removal for 120 h in suckled crossbred cows managed in a pasture-based system. Anim Reprod Sci 2008, **106**, 298-310.
16. **Sá Filho MF, Marques MO, Girotto R, Santos FA, Sala RV, Barbuio JP, Baruselli PS.** Resynchronization with unknown pregnancy status using progestin based timed artificial insemination protocol in beef cattle. Theriogenology 2014, **81**, 284-290.
17. **Sá Filho MF, Torres-Júnior JRS, Penteado L, Gimenes LU, Ferreira RM, Ayres H, Castro e Paula LA, Sales JNS, Baruselli PS.** Equine chorionic gonadotropin improves the efficacy of a progestin-based fixed-time artificial insemination protocol in Nelore (*Bos indicus*) heifers. Anim Reprod Sci 2010, **118**, 182-187.
18. **Sani RN, Farzaneh N, Moezifar M, Seifi HA, Tabatabei AA.** Evaluation of five resynchronization methods using different combinations of PGF2α, GnRH, estradiol and an intravaginal progesterone device for insemination in Holstein cows. Anim Reprod Sci 2011, **124**, 1-6.
19. **Stevenson JS, Johnson SK, Medina-Britos MA, Richardson-Adams AM, Lamb GC.** Resynchronization of estrus in cattle of unknown pregnancy status using estrogen, progesterone, or both. J Anim Sci 2003, **81**, 1681-1692.
20. **Yavas Y, Walton JS.** Postpartum acyclicity in suckled beef cows: a review. Theriogenology 2000, **54**, 25-55.