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Optimizing the use of sex-sorted sperm in timed artificial insemination programs for suckled beef cows[1]

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ABSTRACT: Three experiments were designed to evaluate methods to optimize the use of sex-sorted sperm in timed AI (TAI) programs for suckled beef cows. In all 3 experiments, suckled *Bos indicus* cows were synchronized using an intravaginal progesterone (P4) device during 8 d and a 2.0-mg injection of intramuscular estradiol benzoate (EB) at device insertion. The females received PG and eCG (300 IU) at P4 device removal and 1.0 mg of EB 24 h later. The cows were inseminated 60 to 64 h after P4 device withdrawal. All cows had their ovaries scanned by transrectal ultrasound at TAI to indentify and to measure the largest follicle (LF) present. In Exp. 1, a total of 853 cows had their LF classified as <9 mm or ≥9 mm at the time of TAI; these cows were then randomly assigned to 4 groups according to their LF diameter (<9 mm or ≥9 mm) and the type of sperm used (sex-sorted or non-sex-sorted). There was an interaction ($P = 0.02$) between the type of sperm and LF diameter beginning at TAI[non-sex-sorted ≥9 mm = 58.9%a (126/214); non-sex-sorted <9 mm = 49.5%b (106/214);sex-sorted ≥9 mm = 56.8%ab (134/236); and sex-sorted <9 mm = 31.2%c (59/189), a≠b≠c = $P < 0.05$]. In Exp. 2, suckled cows (n = 491) were classified immediately before TAI as having displayed estrus or not (estrus or no estrus) between P4 device removal and TAI. These cows were randomly assigned to 4 groups according to the occurrence of estrus and the type of sperm (sex-sorted or non-sex-sorted). There were effects of the occurrence of estrus ($P = 0.0003$) and the type of sperm ($P = 0.05$) on pregnancy per AI [P/AI; no estrus, non-sex-sorted = 43.6% (27/62); estrus, non-sex-sorted = 58.5%; (107/183); no estrus, sex-sorted = 33.9% (21/62), and estrus, sex-sorted = 50.0% (92/184)]; however, no interaction between the occurrence of estrus and type of sperm was observed ($P = 0.87$). In Exp. 3, a total of 200 suckled cows presenting LF ≥9 mm at TAI were randomly assigned to receive sex-sorted sperm deposited into the uterine body (n = 100) or into the uterine horn ipsilateral to the recorded LF (n = 100). No effect of deeper AI on P/AI was found ($P = 0.57$). Therefore, the LF diameter at TAI and the occurrence of estrus can be used as selection criteria to identify cows with greater odds of pregnancy to receive sex-sorted sperm in TAI programs. In addition, performing TAI with sex-sorted sperm deeper into the uterus did not alter the pregnancy results.

Key words: *Bos indicus*, cattle, estrus synchronization, sexed sperm, uterus

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INTRODUCTION

Advances in sex sorting of sperm using flow cytometry have enabled its incorporation into commercial reproductive management. Despite the increased use of sex-sorted sperm, pregnancy per AI (**P/AI**) is still less than by conventional semen (DeJarnette et al., 2008). Regardless of these reduced results, longer intervals

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from the induction of ovulation to the AI (i.e., closer to the expected moment of ovulation) have increased the likelihood of pregnancy in females inseminated with sex-sorted sperm (Schenk et al., 2009; Sá Filho et al., 2010a; Sales et al., 2011).

Additionally, uterine horn insemination could be a strategy to improve the P/AI when using sex-sorted sperm (Seidel et al., 1997, 1999; Kurykin et al., 2007). However, most studies have performed the AI with one-half of the semen dose in each horn (Senger et al., 1988; Graves et al., 1991). Therefore, AI with a full dose into the uterine horn ipsilateral to the expected ovulation could be an alternative to improve the P/AI.

Furthermore, the size of the follicle from which ovulation occurs and the occurrence of estrus from progesterone (**P4**) source removal to the TAI have been reported to influence P/AI (Perry et al., 2005, 2007; Sá Filho et al., 2010b, 2011b). Thus, knowledge of the largest follicle (**LF**) diameter at TAI and the occurrence of estrus could be important tools to identify females with a greater likelihood of pregnancy, thereby optimizing the use of sex-sorted sperm in TAI synchronization programs.

The objective of the present study was to evaluate techniques to optimize the use of sex-sorted sperm during TAI programs with suckled beef cows. The hypothesis was that strategies to identify cows with increased odds of pregnancy would improve P/AI when using sex-sorted sperm to values similar to those found when using conventional semen. Additionally, sorted sperm deposition deeper into the uterus would improve the P/AI when compared with sorted sperm deposition in the body of the uterus.

MATERIALS AND METHODS

The present study was approved by the Bioethics Commission of the School of Veterinary Medicine and Zootechny of the University of Sao Paulo (protocol number 1807/2009), and it complied with the ethical principles of animal research.

General Reproductive Management

In all 3 experiments, suckled cows, after calving, were allocated into breeding groups according to calving date. The time of estrus was synchronized in all cows using an estradiol/P4-based TAI protocol. The protocol was initiated between 30 and 60 d postpartum. The cows received an intravaginal device containing 1.0 g of P4 (Exp. 1 and 3 = DIB, Intervet-Shering Plough, Boxmeer, Netherlands and Exp. 2 = Sincrogest, Ouro Fino Animal Health, Sao Paulo, Brazil) plus an intramuscular (**i.m.**) injection of 2.0 mg of estradiol benzoate (EB; Exp. 1 and 3 = Gonadiol, Intervet-Shering Plough and Exp. 2 = Sincrodiol, Ouro Fino Animal Health). Eight days later, the device was removed, and cows received i.m. PGF2α (Exp. 1 and 3 = 112.5 µg of D-cloprostenol, Preloban, Intervet-Shering Plough and Exp. 2 = 375.0 µg of sodium cloprostenol, Sincrocio, Ouro Fino Animal Health) and 300 IU of eCG (Folligon, Intervet-Shering Plough). A second i.m. treatment with 1 mg of EB to induce ovulation was performed 24 h after P4 device removal. Cows were inseminated 60 to 64 h after the P4 device was removed. Frozen-thawed semen was used for all inseminations. All inseminations were conducted by a single technician in all 3 experiments. In all trials, cows remained isolated and were exposed only to Nelore bulls 10 to 15 d after the TAI.

Body condition scores were evaluated on the first day of the estrous synchronization protocol using a 1 to 5 scale (1 = emaciated, 5 = obese; Ayres et al., 2009). The cows had their ovaries examined by transrectal ultrasonography at the time of TAI. The LF present at TAI was identified and measured. All transrectal ultrasound exams were performed by the same technician with a 7.5-MHz linear-array transducer (CTS-3300V, SIUI, Guangdong, China).

Description of the Sorting Procedure

The sorting procedures were performed in the Sexing Technologies facility located in Sertaozinho, SP, Brazil. In all of the experiments, X-bearing sperm were isolated with 85 to 90% purity using a MoFlo SX (Beckman Coulter, Inc., Brea, CA) sperm sorter operated under 241.3 kPa and approximately 40,000 events/s, which resulted in sorting rates ranging from 5,000 to 8,000 sperm/s. Sperm were stained with 112.5 µ*M* Hoechst 33342 at 160×10^6 sperm/mL and sorted at 80×10^6 sperm/mL after filtering at unit gravity through a 50 CellTrics disposable filter (#04-0042-2317; Partec GmbH, Munster, Germany). The sperm were inspected with a laser light at 150 mW.

The semen was extended with 20% egg yolk-Tris extender (6% glycerol; Sexing Technologies, Navasota, TX) and packaged in 0.25-mL polyvinylchloride straws (sex-sorted = 2.1×10^6 and non-sex-sorted = 20×10^6 sperm/straw).

Exp. 1. Effect of the Largest Follicle Diameter at Timed Artificial Insemination Using Sex-Sorted or Non-Sorted Sperm in Suckled Beef Cows

Animals and Synchronization Protocol. A total of 853 suckled, multiparous Nelore *Bos indicus* cows from 2 commercial beef farms, 1 in Jararezinho, PR, and 1 in Campo Grande, MS, Brazil, were used in this study. All cows were kept on a *Braquiaria brizantha* pasture and given mineralized salt and free access to water.

Data collection was performed from September through December of the 2009 breeding season.

Experimental Design. Immediately before insemination, the cows had their LF classified as <9 mm or ≥9 mm into 2 groups (LF <9 mm or ≥9 mm). This LF diameter (9 mm) was previously determined by receiver operating characteristic (**ROC**) analysis performed to determine the critical LF diameter that optimizes P/AI based on sensitivity and specificity (Sá Filho et al., 2011a). Cows in each follicle size category were randomly assigned to be inseminated with sex-sorted sperm (minimum of 2×10^6 cells/inseminating dose) or non-sex-sorted sperm (minimum of 20×10^6 cells/inseminating dose) in a 2 × 2 factorial arrangement of treatments. Cows presenting LF <9 mm at TAI received either non-sex-sorted (n = 214) or sex-sorted (n = 189) sperm, and cows presenting LF ≥9 mm were inseminated with either non-sex-sorted (n = 214) or sex-sorted sperm (n = 236). Semen from 2 Nelore sires was used to inseminate all of the cows and was distributed depending on the farm location or the breeding group from each farm. Both sires used had previously provided satisfactory pregnancy outcomes with sex-sorted sperm in TAI programs. Ejaculates were collected by artificial vagina and contained >65% progressive motile sperm and >85% sperm of normal morphology (<15% primary and <15% secondary morphological abnormalities). Ejaculate was proportionally divided using a split-ejaculate technique to produce the same number of doses of sex-sorted and non-sex-sorted sperm.

Exp. 2. Effect of the Occurrence of Estrus from Progesterone Device Removal to Timed Artificial Insemination Using Sex-Sorted or Non-Sex-Sorted Sperm in Suckled Beef Cows

Animals and Synchronization Protocol. A total of 491 suckled, multiparous Nelore *Bos indicus* cows from 1 commercial beef farm in Ananas, TO, Brazil, were used in this study. All cows were kept on a *Braquiaria brizantha* pasture and given mineralized salt and free access to water. Data collection was performed from November through March of the 2010/2011 breeding season.

Detection of Estrus. Estrus was determined using an adhesive estrus detection aid (Estrotect, IVP, Spring Valley, WI). At P4 device removal, the cows received the estrus detection aid, which was placed between the hips and the tail head. Estrus was determined at TAI by the activation of each device.

Experimental Design. Immediately before the insemination, cows were classified as either having displayed estrus or not (estrus or no estrus), after which they were randomly assigned to be inseminated with sex-sorted sperm (minimum of 2×10^6 cells/inseminating dose) or non-sex-sorted sperm (minimum of 20×10^6 cells/inseminating dose) in a 2 × 2 factorial arrangement of treatments. Estrus cows received either non-sex-sorted (n = 183) or sex-sorted (n = 184) sperm, and the no estrus cows were inseminated with either non-sex-sorted (n = 62) or sex-sorted sperm (n = 62). Semen from 2 Nelore sires was used to inseminate all of the cows and was distributed according to breeding group. Both sires used had previously provided satisfactory pregnancy outcomes with sex-sorted sperm in TAI programs. Ejaculates were collected by artificial vagina and contained >65% progressive motile sperm and >85% sperm of normal morphology (<15% primary and <15% secondary morphological abnormalities). Ejaculate was proportionally divided using a split-ejaculate technique to produce the same number of doses of sex-sorted and non-sex-sorted sperm.

Exp. 3. Effect of the Sex-Sorted Sperm Deposition Site in Suckled Beef Cows

Animals and Synchronization Protocol. A total of 200 suckled, multiparous Nelore *Bos indicus* cows from 1 commercial beef farm in Aruana, GO, Brazil were used in this study. All cows were kept on a *Braquiaria humidicola* pasture and given mineralized salt and free access to water. Data collection was performed from December through March of the 2010/2011 breeding season.

Experimental Design. Immediately before the insemination, the cows had their ovaries examined by transrectal ultrasonography at TAI. The LF present on the ovaries at TAI was identified and measured. Only cows presenting a LF ≥9 mm were considered suitable for the experiment. Immediately after the LF diameter had been recorded, the cows were randomly assigned to receive sex-sorted sperm (minimum of 2×10^6 cells/inseminating dose) deposited into the body (n = 100) or horn (n = 100) of the uterus. Deeper sperm deposition was performed roughly in the proximal two-thirds of the uterine horn ipsilateral to the recorded LF. Semen from the same 2 Nelore sires previously used in Exp. 2 was equally distributed according to experimental group to inseminate all the cows. Ejaculates were collected by artificial vagina and contained >65% progressive motile sperm and >85% sperm of normal morphology (<15% primary and <15% secondary morphological abnormalities).

Pregnancy Diagnosis

In all 3 experiments, pregnancy diagnoses were performed by ultrasound 30 to 42 d after TAI. Detection of an embryonic vesicle with a viable embryo (with the presence of a heartbeat) was used as a positive indicator of pregnancy. Pregnancy per AI was defined as the number of females pregnant 35 to 42 d after AI divided by the total number of females inseminated.

STATISTICAL ANALYSES

Response variables were analyzed using PROC GLIMMIX (SAS Inst. Inc., Cary, NC). Explanatory variables for each cow, such as the cohort of synchronized animals, BCS, treatment, sire, and interactions were included in the initial model. Variables were then removed from the final model by backward elimination (according to Wald's criterion) when $P > 0.10$.

In Exp. 1, the final model for P/AI included variables for the type of sperm (sex-sorted or non-sex-sorted), the LF group (<9 mm or ≥9 mm), and the interaction between the type of sperm and the LF group. In Exp. 2, the final model for P/AI included variables for the type of sperm (sex-sorted or non-sex-sorted) and the occurrence of estrus. In Exp. 3, the final model for P/AI included only the site of sperm deposition (body or horn of the uterus).

The relationship between the diameter of the LF at TAI and the probability of occurrence of estrus (Exp. 2) and the probability of pregnancy in cows inseminated with sex-sorted or non-sex-sorted sperm (Exp. 1 and 2) were determined. Information from individual cows from each experiment was collated into a single data set for statistical analysis. Logistic regression curves were created using the coefficients provided by the interactive data analysis from SAS and the formula $y = \exp(\alpha \times x + b)/[1 + \exp(\alpha \times x + b)]$, where y = success probability of pregnancy or occurrence of estrus; exp = exponential; α = slope of the logistic equation; b = intercept of the logistic equation; and x = diameter of the largest follicle at TAI, mm.

RESULTS

Exp. 1. Effect of the Largest Follicle Diameter at Timed Artificial Insemination Using Sex-Sorted or Non-Sex-Sorted Sperm in Suckled Beef Cows

No effect of cow parity (multiparous vs. primiparous; $P = 0.40$) or BCS ($P = 0.60$) on P/AI was observed. No interaction effects on P/AI were found between parity and either type of sperm ($P = 0.51$) or LF diameter at TAI ($P = 0.66$). Additionally, no interaction effects on P/AI were found between BCS and either type of sperm ($P = 0.59$) or LF diameter at TAI ($P = 0.56$).

There was a significant interaction effect on P/AI ($P = 0.02$) between type of sperm and the LF diameter category (<9 mm or ≥9 mm; Figure 1). For cows with follicles <9 mm, greater ($P = 0.0002$) pregnancy rates were found in those inseminated with conventional sperm when compared with sex-sorted sperm (49.5% vs. 31.2%, respectively). However, no differences ($P = 0.65$) where found between non-sex-sorted and sex-sorted sperm when the cows had follicles ≥9 mm (58.9% and 56.8%, respectively).

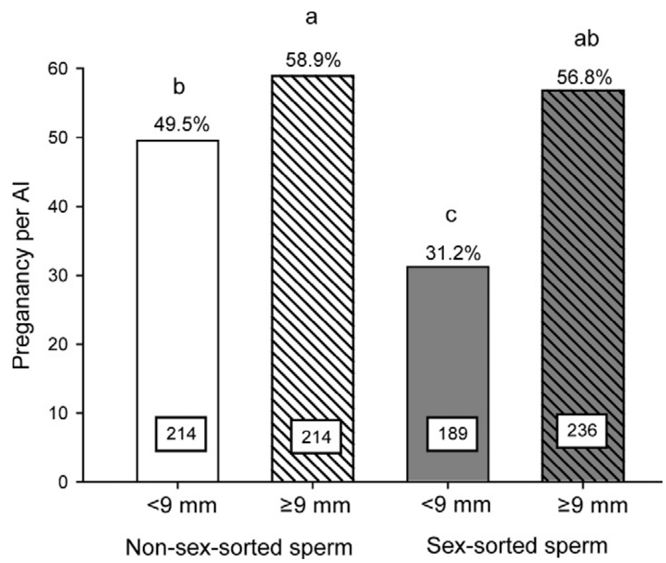

Figure 1. Pregnancy per AI determined by ultrasound 30 to 42 d after timed AI (TAI) in suckled *Bos indicus* cows according to the type of sperm used (sex-sorted or non-sex-sorted sperm) and the largest follicle (LF) diameter (<9 mm or ≥9 mm) identified and measured by ultrasound at TAI. An interaction was observed between the type of sperm and the LF diameter ($P = 0.02$). [a-c] Bars without a common letter differ ($P < 0.05$). Numbers in boxes indicate number of animals bred.

Exp. 2. Effect of the Occurrence of Estrus from Progesterone Device Removal to Timed Artificial Insemination Using Sex-Sorted or Non-Sex-Sorted Sperm in Suckled Beef Cows

Overall frequency of estrus following the TAI protocol was 74.7% (367/491). The cows that presented estrus had a larger diameter (mean ± SEM) of the LF at TAI (11.4 ± 0.19) than those cows that did not present estrus (10.0 ± 0.34; $P = 0.006$). Also, more frequent occurrence of estrus was observed with increasing diameter of the LF at TAI ($P < 0.001$; Figure 2). No interaction ($P = 0.87$) between the type of sperm and the occurrence of estrus was found. Sex-sorted sperm (45.9%; 113/246) resulted in a reduced ($P = 0.05$) P/AI, compared with non-sex-sorted sperm (54.7%; 134/245). Additionally, cows that displayed estrus between the time of P4 device removal and the insemination presented larger ($P = 0.003$) P/AI (54.2%; 199/367) than did cows that had not displayed estrus (38.7%; 48/124) after progestin removal, regardless of sperm type (Figure 3). No effect of BCS on the first day of the synchronization protocol ($P = 0.24$) on P/AI was observed. Additionally, no interaction effects on P/AI were observed between BCS and estrus occurrence ($P = 0.86$) or between BCS plus the type of sperm and estrus occurrence ($P = 0.21$).

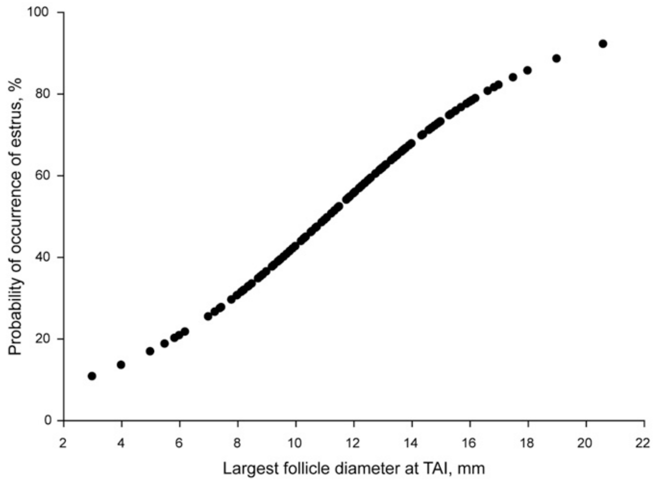

Figure 2. Probability of occurrence of estrus between the intravaginal progesterone device removal and the timed AI (TAI) in suckled *Bos indicus* cows (n = 491) according to the diameter of the largest follicle (LF) identified and measured by ultrasound at TAI [occurrence of estrus = exp (−2.9013 + 0.2601 × diameter of the LF at TAI / 1 + exp (−2.9013 + 0.2601 × diameter of the LF at TAI); $P < 0.0001$)].

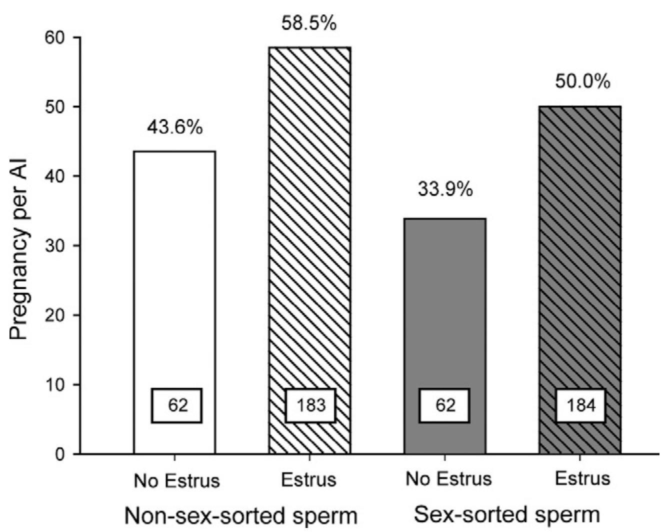

Figure 3. Pregnancy per AI determined by ultrasound 30 to 42 d after the timed AI (TAI) in suckled *Bos indicus* cows according to the type of sperm used (sex-sorted or non-sex-sorted sperm) and the occurrence of estrus (No Estrus or Estrus) between progesterone device removal and the TAI. No interaction between the occurrence of estrus and type of sperm used was observed ($P = 0.87$). A significant effect of the occurrence of estrus ($P = 0.003$) and of the type of sperm ($P = 0.05$) used on pregnancies per AI was found. Numbers in boxes indicate number of animals bred.

The overall pregnancy rate data from Exp. 1 and 2 revealed a significant association ($P < 0.001$) between the probability of pregnancy and LF diameter on the day of insemination, both when using sex-sorted sperm and non-sex-sorted sperm (Figure 4). In addition, the P/AI achieved with the sex-sorted sperm (45.6%; 306/671) was significantly less ($P = 0.0003$) than that with non-sex-sorted sperm (54.4%; 366/673) regardless of the diameter of the LF at TAI. The P/AI achieved after TAI

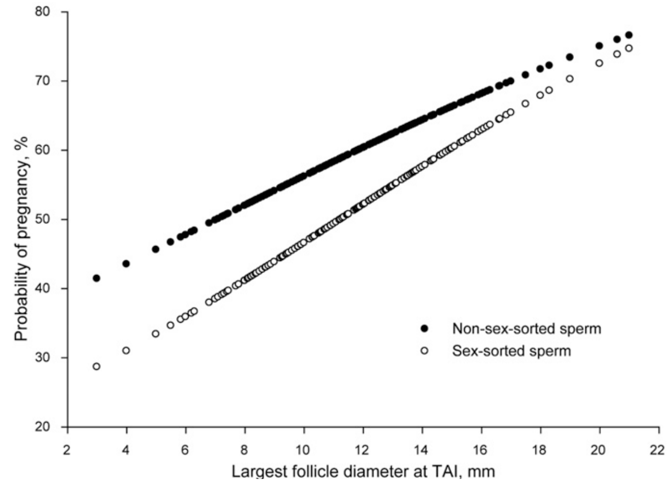

Figure 4. Probability of pregnancy determined by ultrasound 30 to 42 d after timed AI (TAI) in suckled *Bos indicus* cows (n = 1344) according to the type of sperm used [non-sex-sorted sperm (n = 673) or sex-sorted sperm (n = 671)] and the diameter of the largest follicle (LF) identified and measured by ultrasound at TAI [non sex-sorted sperm = exp (−0.6018 + 0.0850 * diameter of the LF at TAI / 1 + exp (−0.6018 + 0.0850 * diameter of the LF at TAI); $P < 0.0001$) or sex-sorted sperm = exponential (−1.2449 + 0.1107 * diameter of the LF at TAI / 1 + exponential (−1.2449 + 0.1107 * diameter of the LF at TAI); $P < 0.0001$)].

using sex-sorted sperm was 83.8% of that achieved with non-sex-sorted sperm.

Exp. 3. Effect of the Sex-Sorted Sperm Deposition Site in Suckled Beef Cows

No difference ($P = 0.93$) was found in the average LF diameter at TAI (Body 12.5 ± 2.7 mm and Horn 12.6 ± 2.2 mm; means ± SD) between the 2 groups. Additionally, insemination with sex-sorted sperm into the body (54.0%; 54/100) of the uterus achieved P/AI ($P = 0.57$) values similar to those achieved with insemination into the horn of the uterus (50.0%; 50/100).

DISCUSSION

This study established 2 practical strategies for optimizing the use of sex-sorted sperm in commercial TAI programs for suckled beef cows. In Exp. 1, greater P/AI values were obtained in cows presenting a LF >9 mm at TAI, mainly when these cows were inseminated with sex-sorted sperm, which agrees with our hypothesis. In Exp. 2, cows that displayed estrus between the time of P4 device removal and the TAI had greater P/AI values than those that did not, regardless of whether they received sex-sorted or non-sex-sorted sperm. No differences, however, were found when the cows were inseminated deeper in the uterus (Exp. 3).

Diameter of the LF at the time of TAI influences ovarian and pregnancy outcomes following TAI protocols in *Bos taurus* and *Bos indicus* cattle (Perry et al., 2005, 2007; Meneghetti et al., 2009; Sá Filho et al., 2009, 2010b; Galvão and Santos 2010). As shown above, in suckled *Bos indicus* cows presenting a larger follicle (≥9 mm), P/AI was similar when using either sex-sorted or non-sex-sorted sperm. According to Vasconcelos et al. (2001), Holstein cows that ovulate small follicles (<13.2 mm) develop smaller corpora lutea, which secreted less P4, when compared with cows induced to ovulate larger follicles (14.4 mm). Another study with *Bos taurus* beef heifers reported that cows that ovulate follicles smaller than 11.3 mm are less likely to support pregnancy to d 60 when compared with heifers that ovulate 14.6-mm follicles (Perry et al., 2007). In *Bos indicus* cows, a positive effect of increased LF diameter at TAI on ovulation and P/AI using non-sex-sorted sperm has also been verified (Meneghetti et al., 2009; Sá Filho et al., 2010b).

Interestingly, cows presenting an LF ≥9 mm at TAI showed enhanced P/AI mostly with sex-sorted sperm, as demonstrated by the significant interaction between the diameter of the LF at TAI and the type of sperm used (Exp. 1). Previous studies demonstrated that greater P/AI could be achieved when a short interval from insemination using non-sex-sorted sperm to ovulation was applied (Hockey et al., 2009), especially using reduced fertility bulls (Macmillan and Watson, 1975). Similarly with sex-sorted sperm, increased P/AI can be reached if the AI is performed closer to ovulation (Schenk et al., 2009; Sá Filho et al., 2010a; Sales et al., 2011). Despite the satisfactory predictability of the moment of ovulation provided by the P4 plus estradiol-based estrus synchronization protocol (averaging 66 to 72 h after P4 device removal), the timing of ovulation is influenced by the diameter of the follicle at the time of the ovulatory stimulus treatment (Neves, 2010). Neves (2010) evaluated the time of ovulation in a large number of suckled *Bos indicus* cows (n = 312) and observed a significant effect of the diameter of the ovulatory follicle on the moment of synchronized ovulation (average of 71.8 ± 7.7 h after P4 device removal). The author reported that cows experiencing premature ovulation (i.e., ovulation occurring from 48 to 59 h after P4 device removal) presented a larger ovulatory follicle (14.0 ± 2.2 mm) than cows with delayed ovulation (11.4 ± 2.2 mm; 73 to 96 h after P4 device removal) and that cows that ovulated at the expected time of ovulation (60 to 72 h after P4 device removal) showed ovulatory follicles of intermediate diameter (13.6 ± 2.1 mm). Therefore, the greater P/AI after AI using sex-sorted sperm observed in cows presenting a larger LF at TAI could be due to greater ovarian responses following the estrous synchronization protocol, or it could also be caused by the short interval between the TAI and the occurrence of ovulation in cows presenting a larger LF diameter at TAI.

Attention should be paid to the difference between *Bos taurus* and *Bos indicus* reproductive physiology regarding the size at which the dominant follicle acquires its ovulatory capacity in response to an ovulatory stimulus (Sartori et al., 2001; Gimenes et al., 2008). In *Bos indicus* cattle, an ovulatory response after porcine LH challenge occurs in 33, 80, and 90% of follicles 7 to 8.4 mm, 8.5 to 10 mm, and > 10 mm in diameter, respectively (Gimenes et al., 2008). However, in Holstein cows (*Bos taurus*), the acquisition of the capacity to ovulate in response to exogenously administered LH only occurs at sizes ≥10 mm (Sartori et al., 2001). Therefore, selecting cows to receive sex-sorted or non-sex-sorted sperm on the basis of the diameter of their LF at TAI is an adjustment to the present strategy that should be considered before application in *Bos taurus* cattle.

In the current study, cows that displayed estrus following the estrous synchronization protocol achieved greater P/AI with sex-sorted sperm than cows that did not display estrus. Also, cows presenting a larger follicle at TAI presented greater P/AI mainly when these cows displayed estrus between the P4 device removal and TAI. Similarly, previous studies have demonstrated that cows that display estrus have a larger follicular diameter at TAI, greater ovulatory responses, and increased subsequent luteal function than cows that do not display estrus (Perry et al., 2005; Hillegass et al., 2008; Sá Filho et al., 2011b). Additionally, greater P/AI with the use of non-sex-sorted sperm has been reported in dairy and beef cows displaying estrus (Perry et al., 2005; Hillegass et al., 2008; Sá Filho et al., 2011b). Thus, the occurrence of estrus could be used to select cows with a greater likelihood of pregnancy to receive sex-sorted sperm, thereby optimizing the commercial use of sexed sperm in TAI reproductive programs for suckled beef cows.

Several studies have been performed to evaluate the effect of uterine horn insemination (Olds et al., 1953; Hawk and Tanabe, 1986; Senger et al., 1988; Williams et al., 1988; McKenna et al., 1990; Graves et al., 1991; Kurykin et al., 2003; Andersson et al., 2004; Verberckmoes et al., 2004). The majority of those previous results support the idea that site of semen deposition would play little to no role in P/AI. However, because of the presumably reduced viability of sexed sperm, the insemination closer to the site of ovulation would potentially provide better results (Pallares et al., 1986; Seidel et al., 1997). Also, it is important to mention that in most of those studies, the cornual inseminations were performed by depositing one-half of the semen straw into the right horn and the other one-half into the left horn. In the current study, the insemination was performed with the full dose in the uterine horn ipsilateral to the expected side of ovulation. Despite these differences, similar P/AI were found when comparing inseminations performed in the horn or in the body of the uterus, which agrees with those previous results with conventional semen.

Overall P/AI rates were reduced with sex-sorted sperm compared with non-sex-sorted sperm (i.e., 83.8% pregnancy was obtained with the non-sex-sorted sperm). These results are similar to those obtained by other authors using sex-sorted sperm in AI programs after estrus detection (DeJarnette et al., 2008) or following TAI protocols (Sales et al., 2011). This reduced P/AI could be attributable to several factors including a shorter lifespan in the female reproductive tract; reduced number of sperm per straw; and sperm damage from the staining, identification, and separation processes (Maxwell et al., 2004; Schenk and Seidel, 2007; DeJarnette et al., 2008; Schenk et al., 2009). It is important to note that all sires used in the present study had previously provided satisfactory pregnancy outcomes in TAI programs either using sex-sorted or non-sex-sorted sperm. Moreover, there are individual bull differences that can significantly affect pregnancy outcomes when sex-sorted sperm is used (DeJarnette et al., 2008). The bull effect can alter the proportion of pregnancies achieved with sex-sorted sperm versus conventional semen (Sales et al., 2011). Thus, the predetermination of sire fertility is still the main issue when sex-sorted sperm is employed in commercial TAI programs.

In conclusion, by simply identifying estrus display and by performing an ultrasound before insemination, suckled beef cows with greater odds of pregnancy can be assigned to receive expensive or sex-sorted sperm in TAI synchronization programs, thereby greatly improving the cost-benefit margin of beef production. In particular, the estrus occurrence information can be practical and easy to record and incorporated into the routine reproductive management of commercial beef farms. In addition, performing TAI with sex-sorted sperm deeper in the uterus did not alter the pregnancy results.

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