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Timing of insemination and fertility in dairy and beef cattle receiving timed artificial insemination using sex-sorted sperm

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Abstract

The objective was to evaluate the effects of timing of insemination and type of semen in cattle subjected to timed artificial insemination (TAI). In Experiment 1, 420 cyclic Jersey heifers were bred at either 54 or 60 h after P4-device removal, using either sex-sorted (2.1×10^6 sperm/straw) or non-sorted sperm (20×10^6 sperm/straw) from three sires (2 × 2 factorial design). There was an interaction ($P = 0.06$) between time of AI and type of semen on pregnancy per AI (P/AI, at 30 to 42 d after TAI); it was greater when sex-sorted sperm ($P < 0.01$) was used at 60 h (31.4%; 32/102) than at 54 h (16.2%; 17/105). In contrast, altering the timing of AI did not affect conception results with non-sorted sperm (54 h = 50.5%; 51/101 versus 60 h = 51.8%; 58/112; $P = 0.95$). There was an effect of sire ($P < 0.01$) on P/AI, but no interaction between sire and time of AI ($P = 0.88$). In Experiment 2, 389 suckled *Bos indicus* beef cows were enrolled in the same treatment groups used in Experiment 1. Sex-sorted sperm resulted in lower P/AI (41.8%; 82/196; $P = 0.05$) than non-sorted sperm (51.8%; 100/193). In addition, there was a tendency for greater P/AI ($P = 0.11$) when TAI was performed 60 h (50.8%; 99/195) versus 54 h (42.8%; 83/194) after removing the progestin implant. In Experiment 3, 339 suckled *B. indicus* cows were randomly assigned to receive TAI with sex-sorted sperm at 36, 48, or 60 h after P4 device removal. Ultrasonographic examinations were performed twice daily in all cows to confirm ovulation. On average, ovulation occured 71.8 ± 7.8 h after P4 removal, and greater P/AI was achieved when insemination was performed closer to ovulation. The P/AI was greatest (37.9%) for TAI performed between 0 and 12 h before ovulation, whereas P/AI was significantly less for TAI performed between 12.1 and 24 h (19.4%) or >24 h (5.8%) before ovulation. In conclusion, sex-sorted sperm resulted in a lesser P/AI than non-sorted sperm following TAI. However, improvements in P/AI with delayed time of AI were possible (Experiments 1 and 3), and seemed achievable when breeding at 60 h following progestin implant removal, compared to the standard 54 h normally used in TAI protocols.

Keywords: Cattle; Ovulation; Time of AI; Sexed sperm; Estrous synchronization

1. Introduction

In the last decade, the use of sex-sorted bovine sperm has been widely incorporated in commercial beef and dairy operations, depending on management strategy and geographic location. The possibility of increasing the production of calves of a desired sex has important economic implications for beef and dairy industries worldwide. For instance, dairy producers can now produce greater numbers of replacement heifers to offset poor fertility and high culling rates in modern

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lactating herds. Likewise, for beef producers, efficiently increasing the proportion of male calves relative to cohort females is economically favorable. As a result, the cattle industry worldwide has become increasingly interested, and in some geographic regions, heavily dependent on sex-sorted sperm.

In general, AI stud companies recommend the use of sex-sorted sperm only in virgin heifers that display strong signs of estrus (standing to be mounted by a herdmate). Although his strategy can produce good results in terms of conception rate [1], it is incompatible with newer trends in reproductive management used in modern dairy and beef operations, e.g., extensive synchronization programs [2]. Currently, several types of GnRH/$PGF_{2\alpha}$ and progesterone/progestin-based estrous synchronization protocols have been developed for use in cattle [3,4], allowing farmers to perform timed AI (TAI) without estrous detection. Therefore, TAI protocols are rapidly replacing regular estrous detection systems in both dairy [5,6] and beef [7] herds, necessitating the development of guidelines for the use of sex-sorted sperm in association with TAI protocols.

To date, several groups have studied the appropriate timing of AI relative to the onset of estrus or ovulation in cows bred with non-sorted sperm [8–12]. The general consensus is that later AI (>12 h after the onset of estrus) usually results in greater fertilization rates but lower embryo quality when compared to insemination closer to the onset of estrus [13,14]. For example, a large field study that included 17 herds and 2,661 breedings demonstrated that inseminating cows with non-sorted sperm >24 h after the onset of estrus resulted in a dramatic reduction in the frequency of pregnancy compared to inseminations performed between 4 and 12 h after the onset of standing estrus [10]. Unfortunately, the optimal interval for AI with non-sorted sperm may not be compatible with the use of sex-sorted sperm for several reasons, including the potentially reduced lifespan of sex-sorted sperm in the female reproductive tract [15], fewer numbers of sorted sperm/straw [16], and possible pre-capacitation induced by the sorting procedure [17]. In a small field trial, Schenk et al [18] reported increased P/AI in heifers receiving AI 18–24 h after the observed onset of estrus, as compared to those inseminated at 0–12 h. It is therefore reasonable to expect that decreasing the insemination-ovulation interval may be critical for achieving greater conception rates with sex-sorted sperm following TAI.

Identifying the proper period in which to perform AI with sex-sorted sperm following synchronization programs will play a major role in improving the fertility of sex-sorted sperm and allow wider utilization of sorted sperm in modern commercial dairy and beef operations. Therefore, the objectives of the present study were to evaluate various intervals for AI with sex-sorted sperm, after removal of progesterone devices from dairy heifers and beef cows, with the hypothesis that increased pregnancy per AI (P/AI) will occur when TAI with sex-sorted sperm is performed closer to synchronized ovulation.

2. Materials and methods

2.1. Experiment 1—Timing of TAI using sex-sorted or non-sorted sperm in Jersey heifers

2.1.1.Animals and management

Experiment 1 was conducted in a large commercial heifer operation in Dalhart, TX, USA from October to December 2008, with Jersey heifers (11 to 14 mo old). Heifers were housed in dry lot corrals and fed (four times daily) a total mixed ration formulated to meet or exceed dietary requirements of dairy heifers [19].

2.1.2. Experimental design

Jersey heifers (n = 420) that had not conceived after two or three inseminations of sex-sorted sperm were enrolled in this experiment. On Day 0, heifers received an intravaginal device containing 1.38 g of progesterone (CIDR, Pfizer Animal Health, New York, NY, USA) and an im injection of 2.0 mg of estradiol benzoate (EB). The EB solution was produced as previously described [20]. On Day 8, all heifers received 25 mg of IM $PGF_{2\alpha}$ (dinoprost tromethamine; Lutalyse, Pfizer Animal Health) at the time of progesterone (P4) device removal, and a second im injection of 1 mg of EB 24 h later, as previously described [21]. Heifers were assigned to receive TAI at either 54 (n = 206) or 60 h (n = 214) after P4 device removal, and were randomly inseminated with either frozen-thawed, sex-sorted sperm (n = 213) or non-sorted sperm (n = 207) in a 2 × 2 factorial arrangement. Thus, approximately half of the heifers inseminated at each time point after P4 device withdrawal received non-sorted sperm (n = 101 for the 54 h group and n = 112 for the 60 h group), and approximately half received sex-sorted sperm (n = 105 for the 54 h group and n = 102 for the 60 h group). In addition, semen from three Jersey sires were distributed among the groups. Ejaculates from each sire were collected by artificial vagina and contained >65% progressively motile and >85% morphologically normal sperm. The semen from each sire was proportionally

divided to produce the same number of doses of sex-sorted and non-sorted sperm.

To estimate the timing of synchronized ovulations, a subset of heifers (n = 53) were examined with transrectal ovarian ultrasonography every 12 h, from P4 device removal to either ovulation or 96 h after the first ultrasonographic examination, whichever occurred first. The ultrasound machine, equipped with a 4.5/8.5 MHz crystal array vet linear transducer (Easi-Scan, BCF Technology, Scotland, UK) was operated by the same technician for all ovarian exams. Ovulation was considered to have occurred at the time of the final scan when a previously observed dominant follicle was no longer present.

2.2. Experiment 2—Timing of TAI using sex-sorted or non-sorted sperm in suckled Nelore beef cows

2.2.1. Animals and management

A total of 389 suckled, multiparous Nelore cows from two commercial beef farms in Parana state, Brazil, were used in this study. All cows were maintained on a *Brachiaria brizantha* pasture and given mineralized-salt and free access to water. Data were collected from November to January of the 2006–2007 breeding season.

2.2.2. Experimental design

Postpartum suckled cows were allocated into breeding groups according to calving date. All cows were synchronized with an estradiol plus progestin-based TAI protocol [4]. Hormonal treatments were initiated between 35 and 65 d postpartum. At unknown stages of the estrous cycle, cows were assigned to the estrous synchronization protocol using an ear implant that contained 3 mg norgestomet and an injection of estradiol valerate (EV; 5 mg im) and norgestomet (3 mg im; Crestar®, Intervet-Schering Plough, Boxmeer, Netherlands). Nine days later, progestin implants were removed, and all cows received 400 IU of equine chorionic gonadotropin im (eCG, Folligon®, Intervet-Schering Plough). Then, 48 h after removal of the progestin implant, all cows received 100 μg of GnRH im (Fertagyl®, Intervet-Schering Plough).

Cows were randomly assigned to be inseminated either 54 (n = 194) or 60 h (n = 195) after progestin implant removal. At TAI, cows were randomly reassigned to be inseminated with non-sorted (n = 193) or sex-sorted sperm (n = 196) in a 2 × 2 factorial arrangement. Thus, cows inseminated at 54 h received either non-sorted (n = 95) or sex-sorted (n = 99) sperm, and the remaining cows were inseminated at 60 h with either non-sorted (n = 98) or sex-sorted sperm (n = 97). Semen from a single Nelore sire was used to inseminate all cows. Ejaculates were collected by artificial vagina and contained >65% progressively motile and >85% morphologically normal sperm. These ejaculates were proportionally divided to produce the same number of doses of sex-sorted and non-sorted sperm.

To evaluate the time of ovulation following progestin implant removal, a random subset of cows (n = 20) received ovarian ultrasonographic examinations every 12 h, starting at progestin implant removal and continuing until either ovulation or 96 h after removal, whichever occurred first. An ultrasound machine with a 4.5/8.5 MHz crystal array vet linear transducer (Easi-Scan, BCF Technology) was operated by the same technician for all reproductive examinations.

2.3. Experiment 3—The effect of timing of insemination relative to ovulation on P/AI in suckled cows

2.3.1. Animals and management

A total of 339 suckled, multiparous Nelore cows from a State Research farm (APTA–Alta Mogiana Regional Center), in Colina, Sao Paulo, Brazil, were used in this study. All cows were kept on a *B. brizantha* pasture and given mineralized-salt and free access to water. Data collection was performed from November through March of the 2009–2010 breeding season.

2.3.2. Experimental design

Postpartum suckled cows were allocated into breeding groups according to calving date. All cows were synchronized using an estradiol/progesterone-based TAI protocol [4]. Hormonal treatments started between 30 and 60 d post-partum. Cows received an intravaginal device containing 1.0 g of P4 (Sincrogest®, Ourofino Agronegocio, Sao Paulo, SP, Brazil) plus an im injection of 2.0 mg of EB (Sincrodiol®, Ourofino Agronegocio). Eight days later, the device was removed and cows received 0.25 mg $PGF_{2\alpha}$ im (cloprostenol sodium; Sincrocio®, Ourofino Agronegocio) and 300 IU eCG im (Folligon®, Intervet-Schering Plough). To induce ovulation, a second treatment of 1.0 mg EB im was given 24 h after P4 device removal.

Cows were randomly assigned to be inseminated with sex-sorted sperm (2.1×10^6 sperm/straw) at 36, 48, or 60 h after P4 device removal. Semen from a single Angus sire was used to inseminate all cows. Ejaculates were collected by artificial vagina and contained >65% progressively motile and >85% morphologically normal sperm. A single straw from each freeze batch was tested immediately after thawing for

motility (0 h), and following post-thaw incubation for 3 h, motility and percentage intact acrosomes were assessed. Minimum post-thaw criteria for semen to be used in the study were >30% progressively motile sperm and 65% intact acrosomes.

The interval between TAI and ovulation was measured in all cows by ultrasonographic examinations every 12 h, starting at the time of progesterone device removal and continuing until ovulation or 96 h later, whichever came first. All transrectal ultrasonographic examinations were performed by the same technician, using a 7.5 MHz linear-array transducer (CTS-3300V, SIUI, Guangdong, China).

2.4. Pregnancy diagnosis

Pregnancy diagnoses were made ultrasonographically 30 to 42 d after TAI. Detection of an embryonic vesicle with a viable embryo (presence of a heartbeat) was used as a positive indicator of pregnancy. Pregnancy per AI (P/AI) was defined as the number of females pregnant 30 to 42 d after AI, divided by the total number of females inseminated.

2.5. Brief description of the sorting procedure

The same sorting procedures were used in the Sexing Technologies facilities located in Navasota, TX, USA and Sertaozinho, SP, Brazil. In all experiments, isolation of X-bearing sperm cells with 85–90% accuracy was accomplished with a MoFlo® SX (DakoCytomation, Sexing Technologies, Navasota, TX, USA) sperm sorter operated under 35 psi and approximately 40,000 events/s, which resulted in sorting rates ranging from 5000 to 8000 sperm/s. Sperm were stained with 112.5 μM 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). Sperm were interrogated with 150 mW of laser intensity.

Semen was extended in a 20% egg yolk-Tris extender (6% glycerol; Sexing Technologies©) and packaged in 0.25 mL polyvinylchloride straws (sex-sorted = 2.1×10^6 and non-sorted = 20×10^6 sperm/straw) containing 0.2 mL total volume, and frozen on racks in static liquid nitrogen vapor according to standard procedures used by Sexing Technologies.

2.6. Statistical analyses

Binomial variables, e.g., P/AI, were analyzed using PROC GLIMMIX from the SAS program (SAS Institute Inc., Cary, NC, USA). Explanatory variables for each cow, such as the cohort of synchronized cattle, body condition score (BCS), treatment, sire, AI technician 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 $P > 0.10$ by multivariate logistic regression using the LOGISTIC procedure in SAS. In Experiment 1, the final model for P/AI included variables for the type of semen (sex-sorted or non-sorted), the time of AI (54 or 60 h after intravaginal device removal), the sire (A, B, or C), and the interaction between the type of semen and the time of AI. In Experiment 2, the final model for P/AI included variables for the type of semen, the time of AI, and the interaction between the type of semen and the time of AI. In Experiment 3, the final model for P/AI included only a variable for the time of AI.

Additional analyses regarding follicular dynamics were performed in subsets of heifers (Experiment 1) or cows (Experiment 2). The models were the same as described above, but also included variables for the diameter of the dominant follicle at the time of progesterone/progestin device removal, the diameter of the ovulatory follicle, the time to ovulation, and the interval from TAI to ovulation. In Experiment 3, an additional analysis was conducted to evaluate the effect of the dominant follicle diameter at 24 h after device removal on the time of ovulation. Data were analyzed using the GLM procedure of SAS, and means were compared using a Tukey test.

In Experiment 3, data were analyzed by multivariate logistic regression using the LOGISTIC procedure of SAS (SAS Inst. Inc.). A backward, stepwise regression model was utilized, and explanatory variables were sequentially removed from the model by the Wald statistic criterion if $P > 0.10$. The statistical model to analyze the effect of the interval from intravaginal device removal to ovulation on P/AI included variables for the cohort of the synchronized cow, the BCS at the first day of the synchronization protocol and the interval from TAI to ovulation. The final model for analysis of the interval between TAI and synchronized ovulation included only the interval between TAI and the synchronized ovulation. An adjusted odds ratio (AOR) and 95% confidence interval (CI) were generated during the logistic regression. Results are presented as proportions and AOR.

3. Results

3.1. Experiment 1—Timing of AI using sex-sorted or non-sorted sperm in Jersey heifers

The incidence of ovulation after the synchronization protocol was 92.5 % (49/53). The average diameter of

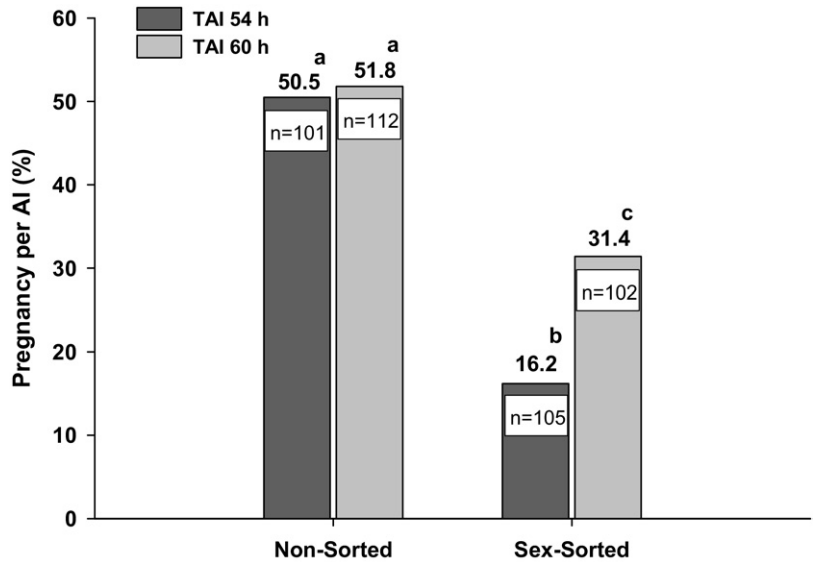

Fig. 1. Pregnancy per AI in Jersey heifers according to the timing of insemination after progesterone device removal (54 or 60 h) and the type of semen used (sex-sorted or non-sorted). There was an interaction between the time of AI and the type of semen (P = 0.06).

[a–c]Bars without a common superscript differed (P < 0.05).

ovulatory follicles was 15.4 ± 0.5 mm, and the interval between P4 device removal and synchronized ovulation was 66.5 ± 4.1 h.

There was an interaction (P = 0.06) between the timing of insemination and the type of semen used (Fig. 1). Specifically, P/AI was greater when sex-sorted sperm (P < 0.01) was used at 60 h (31.4%, 32/102) versus 54 h (16.2%, 17/105) after P4 device removal. The timing of AI did not affect P/AI in heifers bred with non-sorted sperm (54 h = 50.5%, 51/101 vs. 60 h = 51.8%, 58/112; P = 0.95). In addition, there was an effect (P < 0.01) of sire on P/AI (Sire A = 27.3%, 38/139[b]; Sire B = 44.2%, 61/138[a]; Sire C = 41.3%, 59/143[a]), but no interaction between sire and time of AI (P = 0.88), or sire and type of semen (P = 0.16).

3.2. Experiment 2—Time of TAI using sex-sorted or non-sorted sperm in suckled Nelore cows

The incidence of ovulation after the synchronization protocol was 80.0% (16/20). The average diameter of ovulatory follicles was 11.6 ± 0.1 mm, and the interval between removal of the progestin implant and ovulation was 71.3 ± 2.2 h.

There was no interaction between the type of semen and the timing of AI on the P/AI (P = 0.74). Sex-sorted sperm (41.8%; 82/196) resulted in a lower (P = 0.05) P/AI than did non-sorted sperm (51.8%; 100/193). Regardless of semen type, cows inseminated at 54 h (42.8%; 83/194) had similar (P = 0.11) P/AI to cows inseminated at 60 h (50.8%; 99/195) after progestin removal. No significant effects were observed for the farm, AI technician, BCS, or interactions.

3.3. Experiment 3—Timing of insemination relative to ovulation on P/AI in suckled Nelore cows inseminated with sex-sorted sperm

The incidence of ovulation following the synchronization protocol was 92.0% (312/339). The average diameter of ovulatory follicles was 14.7 ± 2.3 mm, and the average interval between P4 device removal and ovulation was 71.8 ± 7.7 h. The distribution of synchronized ovulations relative to the time of device removal was 48 h (6.7%; 21/312), 60 h (0.6%; 2/312), 72 h (80.7%; 252/312), 84 h (11.2%; 35/312), and 96 h (0.6%; 2/312).

The P/AI results were dramatically improved when AI was performed at 60 h (30.9%; 38/123)[a], compared to 48 h (20.8%; 27/130)[b] or 36 h (5.8%; 5/86)[c]. When the time of AI was retrospectively compared to the actual time of ovulation for each cow, greater P/AI were achieved when TAI was performed closer to ovulation (AI >0 to 12 h before ovulation = 37.9%; 36/95) than for other intervals (AI >12 to 24 h before ovulation = 19.4%; 21/108; P = 0.05; or AI >24 h before ovulation = 5.8%; 5/87; P < 0.01; Table 1). In addition, there were no differences in P/AI (P = 0.95) for inseminations performed between 0 to 12 h prior to

Table 1
Risk of pregnancy based on the interval between TAI and ovulation in suckled *B. indicus* cows inseminated with sex-sorted sperm (Experiment 3).

Interval from TAI to ovulation (h)	No. cows	Pregnant (%) No./No.	Adjusted OR[x] (95% CI)	P
> 24	87	5.8 5/87[c]	0.24 (0.08–0.70)	0.01
> 12 to 24	108	19.4 21/108[b]	Reference group[y]	
> 0 to 12	95	37.9 36/95[a]	2.34 (1.22–4.51)	0.01
After ovulation[z]	22	36.4 8/22[ab]	1.80 (0.64–5.03)	0.27

[a,b] Within a column, proportions without a common superscript differed ($P < 0.05$).
[x] OR, odds ratio; CI, confidence interval.
[y] Reference, reference group for adjusted risk ratio, which is the industry standard for the optimal timing of AI with non-sorted sperm.
[z] Inseminations were performed within 0–12 h after ovulation.

ovulation and inseminations performed after ovulation (36.4%; 8/22).

4. Discussion

This study provided insights regarding the importance of the time of insemination when using sex-sorted sperm for TAI in cattle. According to Experiments 1 and 3, the most appropriate time for AI with sex-sorted sperm seemed somewhat later than the industry-recommended guidelines for AI with non-sorted sperm, confirming the initial hypothesis of this study. In contrast, the same hypothesis was not confirmed in Experiment 2. These contrasting results could be due to differing animal category, physiological status, and number of sires used in each experiment.

In the current study, the interval between removal of the progesterone/progestin implant and ovulation ranged from 66 to 72 h and ovulation rate ranged from 80 to 92.5% (Experiments 1, 2 and 3). Previous studies have defined similar intervals between 64 and 76 h and similar ovulation rate for beef cows [21–25]. It is important to acknowledge that this consistent time frame for synchronized ovulations allowed us to characterize the P/AI resulting from various insemination intervals with sex-sorted and non-sorted sperm.

These results, consistent with most of the published literature, indicated that sex-sorted sperm produced lower P/AI than non-sorted sperm. In addition, the P/AI reported here for both sex-sorted and non-sorted sperm were in the same range or slightly lower to P/AI described by previous reports [1,16,26–28]. Thus, further research is needed to confirm and overcome possible physiological reasons for the relatively low P/AI following sexed semen in Jersey heifers observed in Experiment 1. The decreased fertility of sex-sorted sperm has been attributed to several factors, including decreased sperm (2.1 vs 20×10^6 sperm) per insemination dose [29], damage to sperm during the sorting process [30], and potentially reduced viability of sex-sorted sperm in the female genital tract [15,18]. Furthermore, heifer fertility decreases with increasing service number [31], and we must recognize that heifers used in Experiment 1 had previously failed to conceive to two or three previous services.

Interestingly, a delayed AI strategy enhanced the P/AI for sex-sorted sperm, demonstrated by the interaction ($P = 0.06$) between the timing of insemination and the type of semen used (Experiment 1). Additional evidence (Experiment 3) indicated that increased P/AI was achieved with sex-sorted sperm when AI was performed closer to ovulation. Specifically, P/AI was greater when TAI was performed with sex-sorted sperm within 0 to 12 h before synchronized ovulation (Experiments 1 and 3), rather than earlier, as recommended for non-sorted sperm [8–11,13]. These results were consistent with a recent study that inseminations performed within 16 to 24 h after the onset of estrus (i.e., 2 to 10 h before ovulation) were associated with an increased frequency of pregnancy relative to inseminations performed 12 to 16 h after the onset of estrus [32]. Similarly, another report [18] demonstrated that delaying the time of insemination with sex-sorted sperm resulted in either improved or unchanged rates of pregnancy. Differences between Experiments 1 and 2 regarding sex-sorted sperm fertility relative to TAI in heifers and cows further highlighted the difficulties in developing and implementing a dairy heifer TAI protocol.

Dransfield et al [10] evaluated a large number of breedings using the HeatWatch® system, and found the highest fertility after AI with non-sorted sperm occurred within 4 to 12 h after the onset of standing activity (i.e., 23 to 15 h before the predicted time of ovulation). Other large field trials [8] with virgin dairy heifers and non-sorted sperm have demonstrated that optimal conception rates (~60%) occurred when breeding was performed within the first 13 h after observed estrus, whereas breeding performed between 13 h and 33 h after observed estrus yielded lower conception rates (~40%). In addition, lower P/AI were achieved for TAI using non sex-sorted sperm performed closer (4 to 6 h prior) to ovulation [25]. An interval of 6 to 10 h

has been proposed to be necessary for non-sorted sperm to acquire fertilization ability [33–35], and furthermore, oocyte viability in the female genital tract is reduced after 8 h [36]. These somewhat conflicting results between sex-sorted and non-sorted sperm could be due to the potentially decreased lifespan of sex-sorted sperm in the uterine tract [15], or differences in the time required for capacitation, which is believed to be ~6 h for non-sorted sperm [33–35] and/or pre-selection of sperm with an intact acrosome by the sorting procedure [37]. Due to the potential decreased viability of sex-sorted sperm in the uterine tract [15], it is plausible that delayed insemination improved fertilization rates by increasing the number of viable sperm available for fertilization, as described by Saacke [13]. Dalton et al [14] demonstrated that the median number of accessory sperm per oocyte/ovum increased from one accessory sperm in cows that were bred 0 h after the onset of estrus to four accessory sperm when AI was performed 24 h after estrus, and this increase was associated with greater fertilization rates (AI at 0 h = 66% vs AI at 24 h = 82%). In contrast, Saacke [13] and Dalton et al [14] argued that lower quality embryos were produced when AI with non-sorted sperm was performed closer to ovulation. This phenomenon of increased numbers of accessory sperm (higher mean number of accessory sperm) but lower embryo quality was also described by Roelofs et al [11] in a recent study using non sex-sorted sperm for AI performed close to or just after ovulation due to aged oocytes. Interestingly, the results presented here suggest that this phenomenon may not be accurate for AI with sex-sorted sperm. Instead, perhaps delaying AI with sex-sorted sperm resulted in greater fertilization rates while maintaining acceptable embryo quality. The presumptive maintenance of embryo quality when performing AI close to ovulation with sex-sorted sperm could be related to induction of capacitation in the flow cytometer sorting procedure [38]. In fact, recent reports indicated that the majority of failed MOET procedures using sex-sorted sperm were due to decreased fertilization rates and not high percentages of low-quality embryos [39]. Thus, production of substandard embryos may be less of a problem than failures of sex-sorted sperm to successfully fertilize after AI, and the possibility of greater fertilization rates with later AI intervals may occur without an increase in low-quality embryos. Although sex-sorted sperm may require less time for capacitation and may be more readily available for fertilization, perhaps the most important benefit of the sorting procedure is minimization of sperm without intact acrosomes [37].

We were intrigued by the high conception rates observed in Experiment 3, in which cows were inseminated after ovulation, but we hesitate to over-interpret these data due to the small study size (22 animals). There is ample scientific evidence that the oocyte lifespan after ovulation is limited to 6 to 10 h [39], and oocyte aging after this threshold is detrimental to embryo quality. Furthermore, sustained sperm transport following AI after ovulation will also likely lead to oocyte aging prior to fertilization [13]. Therefore, it is almost certain that AI performed after ovulation will produce poor quality embryos [11]. Consequently, AI after ovulation is not recommended.

Another important factor to consider in the timing of insemination with sex-sorted sperm is variation in the fertility of individual bulls. In that regard, there was a significant sire effect on P/AI in Experiment 1. Other studies have described the same influence of sire on P/AI [16,28,40]. Despite variations in fertility among bulls, in reality, none of the sex-sorted sperm performed acceptably in terms of P/AI as a percentage of the P/AI achieved with non-sexed product [41] (Experiment 1: Bull A: 14.7/39.4 = 37%; Bull B: 34.9/52.8 = 66%; Bull C: 21.9/61.4 = 35.6%). It is likely that the effects on P/AI of longer intervals between insemination and ovulation could be more pronounced with sex-sorted sperm. Consequently, more research is needed to confirm whether increasing the interval from progesterone/progestin removal to insemination with sex-sorted sperm is a good strategy for improving P/AI and reducing variability among sires.

In conclusion, increasing the interval between progesterone/progestin device removal and TAI, such that most cattle were bred 0 to 12 h before synchronized ovulation, may improve pregnancy rates for TAI programs using sex-sorted sperm. Furthermore, variation in sire fertility continues to be a reason for inconsistency in conception rates, particularly following the use of sex-sorted sperm in commercial AI programs. Another reason for inconsistency in conception rate, as seen in Experiment 1, may include the use of heifers receiving third and fourth services. Further research with a greater number of heifers focused on TAI at first and second service are needed to adequately describe the potential effect of sire on P/AI following the use of sex-sorted sperm.

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