Anim. Reprod., v.11, n.3, p.217-224, Jul./Sept. 2014

Sex-sorted sperm for artificial insemination and embryo transfer programs in cattle

M.F. Sá Filho[1,5], M. Nichi[1], J.G. Soares[1], L.M. Vieira[1], L.F Melo[3], A. Ojeda[2], E.P. Campos Filho[4], A.H. Gameiro[2], R. Sartori[3], P.S. Baruselli[1,5]

[1]Departamento de Reprodução-VRA, FMVZ-USP, São Paulo, SP, Brazil.
[2]Departamento de Nutrição e Produção Animal-VNP, FMVZ-USP, Pirassununga, SP, Brazil.
[3]Departamento de Zootecnia, ESALQ-USP, Piracicaba-SP, Brazil.
[4]Sexing Technologies, Sertãozinho, SP, Brazil.

Abstract

The selection of offspring from the desired sex can be one of the determining factors to increase the genetic progress and farmer´s profitability in either beef or dairy cattle. In fact, the use of sex-sorted sperm has been applied worldwide combined with artificial insemination (AI) upon estrus detection in heifers. Additionally, several researches have been performed aiming to investigate the use of sex-sorted sperm during timed AI (TAI) programs and for insemination of superstimulated donors for *in vivo* embryo programs. Pregnancy per AI (P/AI) of cyclic heifers inseminated in estrus with sex-sorted sperm has been reported to be approximately 75 to 80% of the P/AI of heifers inseminated with non-sorted sperm. Insemination of superstimulated cows with sex-sorted sperm has been reported to reduce the production of viable embryos. Recently, however, it has been demonstrated that P/AI and embryo production per flushing resulting from AI with sex-sorted sperm may be improved when the time of AI is postponed in relation to the time of AI with non-sorted sperm. The P/AI of non stimulated females and fertilization rates and number of embryos recovered from superstimulated females were increased when AI occurred between 16 and 24 h after the onset of estrus (i.e. 6 to 14 h before ovulation). Nonetheless, despite the improvements achieved in the last decade, there is still a significant individual variability in fertility among bulls that have their sperm sex-sorted. It is critical that the pre-determination of the sire fertility is a paramount when sex-sorted sperm is utilized in commercial AI and ET programs. Thus, the aim of this review is to discuss the main concepts related to the use of sex-sorted sperm in TAI and ET programs, addressing some strategies to increase the efficiency of the technique.

Keywords: bovine, fertility, reproduction, sexed semen, sorting technique.

Introduction

Sex-sorting of sperm cells by flow cytometry is an established method that has been commercially used in cattle (Seidel, 2007; Garner and Seidel, 2008; Rath *et al.*, 2013). This technology is an important tool for the dairy and beef industry, leading to greater supply of replacement heifers and the consequent hastening on genetic gain (De Vries *et al.*, 2008; Chebel *et al.*, 2010). Specific in beef farms, the use of sex-sorted semen could increase the incidence of male calves, product of greater interest due to the increased meat production potential. The separation of sperm bearing X and Y chromosomes is possible due to the differences on the DNA content of these cells (X bearing sperm has about 4% more genetic material than Y bearing sperm) identified by flow cytometry (Johnson, 2000).

The sex sorting process by flow cytometry is the most efficient method to separate X from Y-spermatozoa in a large scale (Garner and Seidel, 2008; Rath *et al.*, 2013; Seidel, 2014). Advances in semen sex sorting have enabled incorporation of this technology into commercial operations (De Vries *et al.*, 2008; Norman *et al.*, 2010). Despite the significant advances in sex-sorting sperm using flow cytometry in cattle, lower pregnancy per AI (P/AI) and reduced *in vivo* embryo production is achieved when compared to the rates obtained with non sex-sorted sperm (Schenk *et al.*, 2006, 2009; Larson *et al.*, 2010; Sales *et al.*, 2011; Soares *et al.*, 2011; Sá Filho *et al.*, 2012; Seidel, 2014). The considerable interest in sex-sorting technology worldwide provides several research opportunities and challenges associated to the use of this product in farms. The aim of this review is to bring into focus a summary of our current understanding on the use of sex-sorted sperm in AI and ET programs, as well as strategies to optimize the efficiency of these combined technologies.

Fertility after the use of sex-sorted sperm in cattle

Despite the advances in sex-sorting of sperm using flow cytometry, lower P/AI is currently observed when compared with conventional semen (DeJarnette *et al.*, 2009, 2010, 2011; Sales *et al.*, 2011; Sá Filho *et al.*, 2012). The P/AI of females inseminated with sex-sorted sperm may be influenced by their reduced lifespan in the uterus (Maxwell *et al.*, 2004), reduced number of sorted sperm per straw (Seidel and Schenk, 2008;

[5]Corresponding author: manoelsa@usp.br; barusell@usp.br
Phone: +55(11)3091-7674; Fax: +55(11)3091-7412
Received: June 8, 2014
Accepted: July 13, 2014

Schenk *et al*., 2009; DeJarnette *et al*., 2011) and bull-related fertility (Frijters *et al*., 2009; Sá Filho *et al*., 2010a; DeJarnette *et al*., 2011; Sales *et al*., 2011). The reduced lifespan of the sex-sorted sperm in the female reproductive tract, due to mitochondria modification and DNA fragmentation, could alter the optimum interval to perform AI relative to ovulation (Maxwell *et al*., 2004; Sá Filho *et al*., 2010a; Gosálvez *et al*., 2011;Sales *et al*., 2011; Rath *et al*., 2013).

In a combination of several experiments, Seidel *et al.* (1999) observed that the P/AI of heifers vary from 40 to 68% and from 67 to 82% in those females inseminated with sex-sorted and non-sex-sorted sperm, respectively. Also, Seidel and Schenk (2008) observed a lower P/AI when using sex-sorted sperm (31 to 42%) than non sex-sorted sperm (43 to 62%). In zebu females (433 heifers and 230 non-suckling cows) inseminated with male-sexed sperm following estrus detection (Dominguez *et al*., 2011), lower P/AI was observed when AI was performed using sex-sorted sperm (38.8%; 131/338) than non sex-sorted sperm (57.9%; 188/325). Despite lower P/AI described in the literature in cattle inseminated using sex-sorted sperm; there is consensus that fertility of heifers inseminated upon estrous detection using sex-sorted sperm is about 70 to 80% of the P/AI obtained following the use of conventional semen (Table 1).

Table 1. Pregnancy per AI (P/AI) of females inseminated with non sex-sorted or sex-sorted sperm and the pregnancy proportion obtained by sex-sorted sperm based on non sex-sorted sperm.

Breed	Category	Pregnancy per AI based on type of semen used in AI		Proportion %	Reference
		Non sex-sorted % (n/n)	Sexed % (n/n)		
Timed Artificial insemination					
Beef	Cows	54.2 (232/428)	45.4 (193/425)	83.7	Sá Filho *et al*., 2012
Beef	Cows	54.7 (134/245)	45.9 (113/246)	83.9	Sá Filho *et al*., 2012
Beef	Cows	51.8 (100/193)	41.8 (82/196)	80.7	Sales *et al*., 2011
Beef	Cows	55.3 (105/190)	40.9 (79/193)	74.0	Sales *et al*., 2011
Dairy	Cows	27.1 (44/162)	13.0 (21/161)	48.0	Souza *et al*., 2006, FMVZ/USP, São Paulo, Brazil, unpublished data
Artificial insemination with estrus detection					
Beef	Heifers	67.6 (96/142)	53.7 (130/242)	79.4	Seidel and Schenk, 2008
Beef	Heifers	67.0 (85/126)	52.6 (129/245)	78.5	Seidel and Schenk, 2008
Beef	Cows and Heifers	57.9 (188/325)	38.8 (131/338)	67.0	Dominguez *et al*., 2011
Dairy	Heifers	60.0 (1375/2292)	38.0 (881/2319)	63.3	DeJarnette *et al*., 2011
Dairy	Cows and Heifers	37.7 (160/426)	22.9 (51/223)	60.7	Mellado *et al*., 2010
Dairy	Heifers	56.0 (30082/53718)	45.0 (17893/39763)	80.3	DeJarnette *et al*., 2009
Dairy	Cows and Heifers	37.4 (34/91)	28.8 (38/132)	77.0	Bodmer *et al*., 2005
Dairy	Cows	46.0 (69/149)	21.0 (33/157)	45.6	Andersson *et al*., 2006
Dairy	Heifers	60.0 (74/124)	46.7 (114/244)	77.8	Seidel and Schenk, 2008
Dairy	Heifers	62.0 (163/263)	42.1 (225/534)	67.9	Seidel and Schenk, 2008
Overall		56.0% (32941/58874)	44.3% (20113/45418)	79.1	

In lactating dairy cows, a recent retrospective study demonstrated that the use of sex-sorted sperm for AI of US Holstein cows (10.8 million AI) was able to achieve mean P/AI about 25% (Norman *et al*., 2010). Andersson *et al*. (2006) reported that the average P/AI was 21% with sex-sorted sperm and 46% with non sex-sorted sperm in dairy cows. Schenk *et al*. (2009) verified that lactating dairy cows achieved ~25% of P/AI when using sex-sorted sperm and ~37% with non sex-sorted sperm. Other recent study (DeJarnette *et al*., 2010), evaluated the use of different doses of sex-sorted sperm and non sex-sorted sperm in lactating dairy cows. The P/AI of lactating cows were 23, 25, and 32% following the use of 2.1 and 3.5 × 10^6 sex-sorted sperm dosages and 15 × 10^6 conventional, respectively. Also, in other recent study working with crossbred *Bos indicus* x *Bos taurus* lactating dairy cows, Sá Filho *et al.* (2013) reported lower P/AI in cows receiving AI using sex-sorted sperm following TAI (21.4%) than cows bred upon estrus detection (31.7%).

In brief, the P/AI observed following the use of sex-sorted sperm is dependent on the P/AI normally observed following the use of conventional semen. Thus, similarly to what is observed when conventional semen is used, P/AI of females inseminated with sex-sorted semen is dependent on fertility of the bulls, animal categories (lactating cows or cyclic heifers), and management across different farms. Consequently, the major commercial recommendation for the use of sex-sorted sperm still has been in heifers after detection of estrus, especially due to their higher fertility (DeJarnette *et al*., 2009; Norman *et al*., 2010; Healy *et al*., 2013).

Improving the P/AI by adjusting the time of insemination

The optimal time at which insemination should take place relative to ovulation depends primarily on the lifespan of spermatozoa and the viability of the oocyte in the female genital tract (Hunter and Wilmut, 1984). Dransfield *et al.* (1998) and Roelofs *et al.* (2006) demonstrated that the probability of P/AI decreased when AI using non sex-sorted sperm is performed closer to the moment of ovulation. According to Roelofs *et al.* (2006), fertilization drastically decreases when AI with conventional semen occurs after ovulation.

Our research group performed a study to evaluate different times to perform AI using sex-sorted sperm. Thereby, Jersey heifers (n = 638) were inseminated following estrus detection using radio telemetry (Heat Watch®) in different intervals from onset of estrus to insemination (12 to 16 h; 16 to 20 h; 20 to 24 h and 24 to 30 h) . The P/AI of heifers inseminated from 12 to 16 h after the onset of estrus (37.7%; 40/106) was lower (P = 0.03) than those inseminated from 16.1 to 20 h (51.8%; 85/164) and 20.1 to 24 h (55.6%; 130/234). No differences were observed on P/AI for heifers inseminated from 24.1 to 30 h (45.5%; 61/134) when compared to the other interval groups.

Therefore, increasing the interval from the onset of estrus to AI may increase pregnancy rates when using sex-sorted semen. This could be achieved by increasing the frequency of estrus detection or using methods that allow continuous monitoring of cow activity, e.g. mount monitoring systems. Furthermore, it is important to note that the effect of timing of insemination on pregnancy rate could be more pronounced when using sex-sorted sperm from bulls less tolerant to the sorting process.

In Brazil, it has been recently reported that almost 60% of the AI performed in this country are made at fixed time (Baruselli *et al*., 2012). For this, the use of a P4/progestin plus E2 based TAI protocols has been the most commercially used type of fixed-time synchronization protocol (Baruselli *et al*., 2012). In these ovulation synchronization protocols, an intravaginal device containing P4 or an ear implant containing norgestomet and estradiol benzoate (EB; 2mg i.m.) are administered on day 0; an injection of prostaglandin (PG) F2α on day 8 or 9 at the moment of device withdrawal plus 300 to 400 IU of equine chorionic gonadotropin (eCG). Different ovulation inducers with similar efficiency could be used such as estradiol cipionate (EC; 0.5 mg i.m.) at moment or EB (1mg i.m.) 24 h after the P4/progestin implant removal. Timed artificial insemination usually is performed from 48 to 54 h after P4/progestin source removal (Baruselli *et al*., 2012). A possibility to improve P/AI following the use of sex-sorted sperm is to control the variation in the time of ovulation through the use of ovulation synchronization protocols. For instance, in beef and dairy females, P4/E2 based synchronization protocols induce ovulation 70-72 h after the P4 device removal (Souza *et al*., 2009; Sales *et al*., 2011; Baruselli *et al*., 2012).

Because sex-sorted sperm presents lower viability on the reproductive tract than conventional semen (Maxwell *et al*., 2004), our research group has evaluated P/AI following delayed AI using sex-sorted sperm in heifers. In a first study, Sales *et al.* (2011) inseminated 420 cyclic Jersey heifers at either 54 or 60 h after P4 device removal, using either sex-sorted (2.1 million of sperm) or non sex-sorted sperm (20 million of sperm) from three sires. The interaction between time of AI and type of semen tended (P = 0.06) to affect P/AI. Delayed insemination improved P/AI only when sex-sorted sperm was used (TAI 54 h = 16.2%; 17/105 *vs*. TAI 60 h = 31.4%; 32/102). In contrast, altering the timing of AI did not affect P/AI with non sex-sorted sperm (TAI 54 h = 50.5%; 51/101 *vs*.TAI 60 h = 51.8%; 58/112). Based on these results, Sales *et al.* (2011) used the same experimental design in suckled *Bos indicus* cows. Timing of AI did not improve P/AI of cows receiving sex-sorted semen and the interaction between time of AI and type of semen did not affect P/AI [Non sex-sorted TAI 54 h = 48.4% (n = 95) *vs*. Non sex-sorted TAI 60 h = 55.1% (n = 98) and Sex-sorted TAI 54 h = 37.4% (n = 99) *vs*. Sex-sorted TAI 60 h = 46.4% (n = 97)]. Finally, the same authors evaluated the moment of insemination using sex-sorted sperm relative to the moment of ovulation in suckled *Bos indicus* cows (n = 339). In this study, cows were randomly assigned to receive TAI with sex-sorted sperm at 36, 48, or 60 h after P4 device removal. Ovarian ultrasound examinations were performed twice daily in all cows to verify the moment of ovulation. Ovulation occurred, on average, 71.8 ± 7.8 h after P4 removal, and greater P/AI was achieved when insemination was performed closer to ovulation. Higher P/AI (37.9%, 36/95) was observed for TAI performed between 0 and 12 h before ovulation, whereas P/AI was significantly lower for TAI performed between 12.1 and 24 h (19.4%, 21/108) or >24 h (5.8%, 5/87) before ovulation.

Therefore, improvement on P/AI with delayed time of AI is possible (Table 2), and seemed achievable when breeding is performed 60 h after progestin implant removal compared with the standard 54 h normally used in TAI protocols.

Table 2. Influence of the AI moment, diameter of the largest follicle (LF) and presence of corpus luteum (CL) on the pregnancy rate of heifers and cows submitted to synchronization of ovulation protocols.

Reference	Animal category	Pregnancy per AI % (n/n)		P value
		Early AI time	Late AI time	
Schenk *et al.* (2009)	Angus heifers	34.4 (11/32)	48.6 (17/35)	>0.10
Neves (2010)	Nelore cows	20.8 (27/130)	30.9 (38/123)	<0.05
Sales *et al.* (2011)	Nelore cows	42.8 (100/193)	50.8 (99/195)	0.11
Sales *et al.* (2011)	Jersey heifers	16.2 (17/105)	31.4 (32/102)	<0.01
Overall (early and late AI time)		33.7 (155/460)	40.9 (186/455)	<0.01

Targeted use of sex-sorted sperm in females with a greater likelihood of pregnancy

The size of the dominant follicle at the end of the synchronization of ovulation protocol and the occurrence of estrus from progesterone source removal to the TAI have been reported to influence P/AI (Perry *et al.*, 2005, 2007; Sá Filho *et al.*, 2010b, 2011, 2012). Thus, the targeted use of sex-sorted sperm in females presenting larger follicle (LF) diameter at TAI and those displaying estrus following the synchronization protocol (e.g. greater likelihood of pregnancy) could be important tools to optimize the use of sex-sorted sperm in TAI synchronization programs.

To evaluate the LF diameter at TAI and the occurrence of estrus following TAI programs as the selective criteria to use sex-sorted sperm, Sá Filho *et al.* (2012) performed two trials using suckled *Bos indicus* cows. In the first trial, the authors showed an interaction between the type of sperm and LF diameter at the time of 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; [abc]P < 0.05). In the second trial, suckled zebu cows inseminated with sex-sorted sperm (45.9%, 113/246) presented lower P/AI than those receiving non sex-sorted sperm (54.7%, 134/245). However, recent study (Thomas *et al.*, 2014) indicated an alternative approach for the sex-sorted semen use. The authors showed that when using sex-sorted semen with delayed AI by 20 h from the standard FTAI among cows that failed to express estrus, pregnancy rates (36%) were comparable (P = 0.9) to those achieved using conventional semen at the standard time (37%). Though, different than beef cattle, the strategy to select most fertile dairy cows has presented conflicting results. In a recent study with lactating dairy cows synchronized with the Ovsynch protocol, Karakaya *et al.* (2014) compared the P/AI after TAI with sex-sorted sperm (n = 148) or non sex-sorted sperm (n = 154) when only cows with a follicle size between 12 and 18 mm and clear vaginal discharge at the time of AI were inseminated. The P/AI was lower for cows inseminated with sex-sorted sperm (25.7%) compared with non sex-sorted sperm (39.0%). The authors suggested that, in lactating dairy cows, sex-sorted sperm produced lower fertility results when compared to conventional semen, even when using some selection criteria for the most potentially fertile cows.

Therefore, at least for beef cattle, 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. However, additional studies are required to precisely evaluate these strategies in lactating dairy cows.

Use of sex-sorted sperm in embryo transfer programs

The determination of factors that alter the embryo quality in superovulated cows could determine improvements in ET programs (Santos *et al.*, 2008; Sartori *et al.*, 2009), and provide tools to overcome the low fertility under certain physiological conditions (Baruselli *et al.*, 2009, 2011; Stewart *et al.*, 2011).

The possibility to choose the sex of the offspring in a herd in species of economic interest is a much desired goal in animal production. The benefits of early identification of sex in the acceleration of the genetic progress, when associated with AI and/or ET techniques, were reported by some authors (Taylor *et al.*, 1985).

Although the pre-implantation diagnosis aiming to identifying the sex of bovine embryos by PCR, both produced *in vivo* and *in vitro,* has been consolidated for human with no deleterious influence on conception (Hasler *et al.*, 2002), the ideal method of controlling the sex ratio appears to be the sex-sorting sperm, through the separation of X-bearing sperm that from Y-bearing sperm. Once fully developed, this technique would allow selecting the sex by AI or other biotechnologies, with a much higher impact on animal production than the identification of embryonic sex, as this does not increase the number of embryos of the desired sex (Sartori *et al.*, 2004).

One of the first studies to evaluate the feasibility of using sex-sorted sperm in superovulation programs was conducted by Sartori *et al.* (2004). The advantage of this study was the use of equal amounts of sperm by treatment (sex-sorted sperm or non sex-sorted). At the end of the superovulation protocol, Holstein heifers were randomly allocated to one of three treatments and inseminated once with sex-sorted sperm containing 20 million of sperms 12 h after estrus detection (S20-1X); twice with sex-sorted sperm containing 10 million of sperms, 12 and 24 h after estrus detection (S10-2X); and twice with non sex-sorted sperm containing 10 million of sperms, 12 and 24 h after estrus (NS10-2X). When sex-sorted sperm was used, number and percentage of fertilized and viable embryos recovered per flush was similar between the S20-1X and S10-2X group, but lower than in the U10-2X group. In addition, heifers bred with X-sorted sperm had an increase in the percentage of degenerate embryos when only fertilized structures were included in the analysis (Table 3). Differently, Peippo *et al.* (2009) observed no differences in superstimulated Holstein heifers on the number of embryos/ova per flushing (6.4 ± 4.2 *vs.* 8.6 ± 6.5; Mean ± SD), percentage of transferable embryos (53.9 *vs.* 65.6%) and percentage of unfertilized structures (21.1 *vs.* 10.6%) for sex-sorted and non sex-sorted sperm, respectively. In superstimulated cows inseminated with sex-sorted sperm, no differences were observed in the number of embryos/ova per flushing (10.4 ± 9.6 *vs.* 9.4 ± 6.7; Sex-sorted and non sex-sorted sperm, respectively). However, marked differences were demonstrated in the percentage of transferable embryos (21.1 *vs.* 64.5%) and the percentage of unfertilized structures (56.0 *vs.* 14.4%) for sex-sorted and non sex-sorted sperm, respectively. It is noteworthy that in this study, the dose of conventional semen was 15 million of sperm and of sex-sorted sperm was 2.1 million of sperm. Moreover, the females were inseminated up to 4 times (9 to 15 h intervals), for a total range of 6 to 8 million of sex-sorted sperm and 30 to 45 million of non sex-sorted sperm. Soares *et al.* (2011) conducted a study that involved two experiments that evaluated different times of insemination (12 and 24 h or 18 and 30 h after ovulation induction) and two types of sperm (sex-sorted = 4.2 million of sperm/AI or non sex-sorted = 40 million of sperm/AI) in Nelore (*Bos indicus*) and Holstein (*Bos taurus*) superstimulated cows. The aim was to evaluate the delay in 6 h in the insemination time of the donors, which occurred at a fixed time after induction of ovulation with porcine LH (pLH) administration. For this study, one single Nelore bull produced ejaculates that were proportionally divided to produce sex-sorted and non-sorted sperm that were used for all of the inseminations. The results of the experiment in Nelore cows are presented in the Table 4.Table 5 summarizes the results of the experiment performed in Holstein donor cows. The delay of TAI from 12/24 h to 18/30 h after pLH administration increased the number of embryos produced in superstimulated cows inseminated with sex-sorted sperm. However, the results are still lower than those obtained with the use of non sex-sorted sperm, especially when analyzing the outcomes in Nelore cows. These data suggest the possibility of increasing the efficiency of embryo production with sex-sorted sperm by delaying the TAI in 6 h after pLH treatment for ovulation induction.

Table 3. Results (mean ± SE) of superovulated Holstein heifers inseminated with sex-sorted sperm X (S20-1X: 20 x 10^6 or S10-2X: 10 million of sperm/dose), or non sex-sorted sperm (NS10-2X: 10 million of sperm/dose).

	S20-1X (n = 12)	S10-2X (n = 13)	NS10-2X (n = 14)
No of CL at flushing	15.3 ± 1.7	18.1 ± 3.4	14.1 ± 1.5
Structures/ flushing	6.8 ± 1.6^a	8.9 ± 1.8	9.9 ± 1.9^b
Fertilized/ flushing	3.8 ± 0.8^a	4.9 ± 0.9^a	8.7 ± 1.7^b
% Fertilized/ flushing	63.5 ± 9.2^a	61.9 ± 6.3^a	90.9 ± 4.0^b
Viable/ flushing	1.9 ± 0.7^a	2.3 ± 0.6^a	6.3 ± 1.2^b
% Viable/ flushing	24.3 ± 8.5^a	30.8 ± 7.7^a	71.3 ± 7.3^b
Degenerated/ flushing	1.8 ± 0.4	2.6 ± 0.6	2.4 ± 0.9
% Degenerated/ flushing	39.2 ± 10.2	31.1 ± 5.6	19.6 ± 4.8
% Degenerated/ fertilized	58.6 ± 11.8^a	53.1 ± 9.8^a	24.2 ± 6.4^b

Different superscripts in row indicate difference for $P < 0.05$.

Table 4. Embryo production (mean ± SE) of superovulated Nelore (*Bos indicus)* cows timed artificial inseminated with sex-sorted and non sex-sorted sperm according to the insemination moment.

	Treatments			P
	Non sex-sorted 12 and 24 h	Sex-sorted 12 and 24 h	Sex-sorted 18 and 30 h	
Number of cows	17	18	19	
Total ova/embryos	8.0 ± 3.2	7.1 ± 3.3	9.0 ± 3.8	0.14
Transferable embryos	6.8 ± 2.6^a	2.4 ± 1.8^c	4.5 ± 3.0^b	< 0.001
Percentage of transferable embryos^d	86.1 ± 11.9^a	37.3 ± 26.7^c	48.2 ± 25.9^b	< 0.001
Freezable embryos	6.0 ± 2.4^a	2.0 ± 1.4^c	3.7 ± 2.8^b	< 0.001
Percentage of freezable embryos^e	76.3 ± 19.2^a	31.8 ± 24.5^c	38.0 ± 26.5^b	< 0.001
Degenerate embryos	0.7 ± 0.7	0.9 ± 1.6	1.6 ± 2.1	0.05
Unfertilized oocytes	0.5 ± 0.7^a	3.7 ± 3.6^b	2.9 ± 2.6^b	< 0.001

^{a,b,c}Rows with different superscripts indicate $P < 0.05$; ^{d}Percentage of transferable embryos based on the number of ova/embryos recovered; ^{e}Percentage of freezable embryos based on the number of ova/embryos recovered.

Table 5. Embryo production (mean ± SE) of superovulated Holstein (*Bos taurus)* cows timed artificial inseminated with sex-sorted and non sex-sorted sperm according to the insemination moment.

	Treatments			P
	Non sex-sorted 12 and 24 h	Sex-sorted 12 and 24 h	Sex-sorted 18 and 30 h	
Number of cows	11	11	11	
Total Ova/embryos	10.4 ± 3.4	11.3 ± 4.4	12.4 ± 3.8	0.40
Transferable embryos	8.7 ± 2.8[a]	4.6 ± 3.0[b]	6.4 ± 3.1[ab]	0.007
Percentage of transferable embryos[d]	85.9 ± 14.0[a]	40.7 ± 21.3[c]	54.2 ± 23.2[b]	<0.001
Freezable embryos	6.9 ± 1.8[a]	3.2 ± 1.8[b]	5.4 ± 3.4[ab]	0.007
Percentage of freezable embryos[e]	69.9 ± 16.8[a]	29.9 ± 15.5[c]	45.3 ± 26.6[b]	<0.001
Degenerate embryos	0.7 ± 0.9	1.4 ± 1.8	1.3 ± 1.7	0.43
Unfertilized oocytes	0.9 ± 1.4[a]	5.2 ± 3.1[b]	4.6 ± 2.6[b]	0.0003

[a,b,c]Rows with different superscripts indicate P < 0.05; [d]Percentage of transferable embryos based on the number of ova/embryos recovered; [e]Percentage of freezable embryos based on the number of ova/embryos recovered.

In another study performed by Baruselli *et al.* (2007), superstimulated Nelore cows were timed artificially inseminated using sex-sorted sperm (4.2 million of sperm per TAI) and non sex-sorted sperm (40 million of sperm per TAI) 12 and 24 h after the ovulation induction with GnRH. Donors inseminated with sex-sorted sperm showed lower number of transferable and freezable embryos, higher number of unfertilized embryos and reduced rate of transferable and freezable embryos (Table 6).

Table 6. Embryo production (mean ± SE) of superovulated Nelore (*Bos indicus)* cows timed artificial inseminated with sex-sorted or non sex-sorted sperm.

	Non sex-sorted	Sex-sorted	P
Number of cows	10	10	
Total structures	9.9 ± 0.78	8.4 ± 1.40	0.28
Transferable embryos (Grades 1, 2 and 3)	6.8 ± 0.66	4.2 ± 0.74	0.03
Freezable embryos (Grades 1 and 2)	5.9 ± 0.71	3.5 ± 0.65	0.03
Unfertilized oocytes	1.5 ± 0.48	3.7 ± 0.88	0.01
Degenerates	1.6 ± 0.37	0.5 ± 0.16	0.04
Transferable embryos rate (%)	68.7 ± 6.3	50.0 ± 5.1	0.01
Freezable embryos rate (%)	59.6 ± 5.1	41.7 ± 5.2	0.02

The real practical sense in using this biotechnology is closely related to the generation of offspring in satisfactory quantities and costs. There are few studies that reported the pregnancy per ET (P/ET) in their results or that are designed to find this answer, possibly because of the difficulty in reaching large number of embryos in superovulation programs. Schenk *et al.* (2006) observed no difference in the pregnancy rate of embryos derived from sex-sorted or non sex-sorted sperm. However, in this study, the number of transferred embryos was small. In an experiment performed by Baruselli *et al.* (2007), part of the embryos were transferred immediately after collection (fresh) at fixed time into synchronized recipients (Table 7). Similar P/ET were observed at 30 and 60 days of gestation after transfer of embryos produced with sex-sorted or non sex-sorted semen. After sexing by ultrasound, it was observed that sex-sorted semen resulted in 90.0% females and conventional semen resulted in 52.7% females.

Table 7. Pregnancy rate at 30 and 60 days after embryo transfer from sex-sorted or non sex-sorted sperm and percentage of females at fetal sexing.

	Pregnancy 30 days (%)	Pregnancy 60 days (%)	Fetal sexing (% females)
Non sex-sorted sperm	57.3 (39/68)	52.9 (36/68)	52.7 (19/36)
Sex-sorted sperm	57.1 (24/42)	47.6 (20/42)	90.0 (18/20)

Final considerations

It is possible to obtain acceptable P/AI (~75-80% to those obtained with non-sex sorted sperm) after AI upon estrus detection with sexed semen in cyclic heifers. The adjustment in the moment to perform the AI using sex-sorted sperm, closer to the expected moment of ovulation, improves reproductive outcome in terms of P/AI or embryo production per flushing. Nonetheless, despite the improvements achieved in the last decade, the major concern with the use of sex-sorted semen is related to low fertility and the dramatic individual variability in fertility among bulls undergoing sexing process. The commercial applicability of this technique depends on the establishment of a methodology that minimizes the sperm loss during the sex sorting process, with no deleterious influence on the fertilizing potential. Also, an increased resistance to the cryopreservation process would be desirable.

References

Andersson M, Taponen J, Kommeri M, Dahlbom M. 2006. Pregnancy rates in lactating Holstein-Friesian cows after artificial insemination with sexed sperm. *Reprod Domest Anim*, 41:95-97.

Baruselli PS, Souza AH, Martins CM, Gimenes LU, Sales JNS, Ayres H, Andrade AFC, Raphael CF, Arruda RP. 2007. Sêmen sexado: inseminação artificial e transferência de embriões. *Rev Bras Reprod Anim*, 31:374-381.

Baruselli PS, Ferreira RM, Filho MFS, Nasser LFT, Rodrigues CA, Bó GA. 2009. Bovine embryo transfer recipient synchronisation and management in tropical environments. *Reprod Fertil Dev*, 22:67-74.

Baruselli PS, Ferreira RM, Sales JNS, Gimenes LU, Sá Filho MF, Martins CM, Rodrigues CA, Bó GA. 2011. Timed embryo transfer programs for management of donor and recipient cattle. *Theriogenology*, 76:1583-1593.

Baruselli PS, Sales JN, Sala RV, Vieira LM, Sá Filho MF. 2012. History, evolution and perspectives of timed artificial insemination programs in Brazil. *Anim Reprod*, 9:139-152.

Bodmer M, Janett F, Hässig M, Daas ND, Reichert P, Thun R. 2005. Fertility in heifers and cows after low dose insemination with sex-sorted and non-sorted sperm under field conditions. *Theriogenology*, 64:1647-1655.

Chebel RC, Guagnini FS, Santos JEP, Fetrow JP, Lima JR. 2010. Sex-sorted semen for dairy heifers: effects on reproductive and lactational performances. *J Dairy Sci*, 93:2496-2507.

De Vries A, Overton M, Fetrow J, Leslie K, Eicker S, Rogers G. 2008. Exploring the impact of sexed semen on the structure of the dairy industry. *J Dairy Sci*, 91:847-856.

DeJarnette JM, Nebel RL, Marshall CE. 2009. Evaluating the success of sex-sorted semen in US dairy herds from on farm records. *Theriogenology*, 71:49-58.

DeJarnette JM, Mccleary CR, Leach MA, Moreno JF, Nebel RL, Marshall CE. 2010. Effects of 2.1 and 3.5 × 106 sex-sorted sperm dosages on conception rates of Holstein cows and heifers. *J Dairy Sci*, 93:4079-4085.

DeJarnette JM, Leach MA, Nebel RL, Marshall CE, McCleary CR, Moreno JF. 2011. Effects of sex-sorting and sperm dosage on conception rates of Holstein heifers: is comparable fertility of sex-sorted and conventional semen plausible? *J Dairy Sci*, 94:3477-3483.

Dominguez JHE, Costa DS, Jojot Centurion V, Faria FJC. 2011. Pregnancy rate of Nelore females inseminated with male-sexed semen. *Anim Reprod Sci*, 129:127-131.

Dransfield MBG, Nebel RL, Pearson RE, Warnick LD. 1998. Timing of insemination for dairy cows identified in estrus by a radiotelemetric estrus detection system. *J Dairy Sci*, 81:1874-1882.

Frijters ACJ, Mullaart E, Roelofs RMG, Van Hoorne RP, Moreno JF, Moreno O, Merton JS. 2009. What affects fertility of sexed bull semen more, low sperm dosage or the sorting process? *Theriogenology*, 71:64-67.

Garner DL, Seidel GE Jr. 2008. History of commercializing sexed semen for cattle. *Theriogenology*, 69:886-895.

Gosálvez J, Ramirez MA, López-Fernández C, Crespo F, Evans KM, Kjelland ME, Moreno JF. 2011. Sex-sorted bovine spermatozoa and DNA damage: II. Dynamic features. *Theriogenology*, 75:206-211.

Hasler JF, Cardey E, Stokes JE, Bredbacka P. 2002. Nonelectrophoretic PCR-sexing of bovine embryos in a commercial environment. *Theriogenology*, 58:1457-1469.

Healy AA, House JK, Thomson PC. 2013. Artificial insemination field data on the use of sexed and conventional semen in nulliparous Holstein heifers. *J Dairy Sci*, 96:1905-1914.

Hunter RH, Wilmut I. 1984. Sperm transport in the cow: peri-ovulatory redistribution of viable cells within the oviduct. *Reprod Nutr Dev*, 24:597-608.

Johnson LA. 2000. Sexing mammalian sperm for production of offspring: the state-of-the-art. *Anim Reprod Sci*, 60/61:93-107.

Karakaya E, Yilmazbas-Mecitoglu G, Keskin A, Alkan A, Tasdemir U, Santos JEP, Gumen A. 2014. Fertility in dairy cows after artificial insemination using sex-sorted sperm or conventional semen. *Reprod Domest Anim*, 49:333-337.

Larson JE, Lamb GC, Funnell BJ, Bird S, Martins A, Rodgers JC. 2010. Embryo production in superovulated Angus cows inseminated four times with sexed-sorted or conventional, frozen-thawed semen. *Theriogenology*, 73:698-703.

Maxwell WMC, Evans G, Hollinshead FK, Bathgate R, De Graaf SP, Eriksson BM, Gillan L, Morton KM, O'Brien JK. 2004. Integration of sperm sexing technology into the ART toolbox. *Anim Reprod Sci*, 82/83:79-95.

Mellado M, Coronel F, Estrada A, Ríos FG. 2010. Fertility in Holstein × Gyr cows in a subtropical environment after insemination with Gyr sex-sorted semen. *Trop Anim Health Prod*, 42:1493-1496.

Neves KAL. 2010. *Effect of interval between insemination and ovulation in conception rates in Nelore cows timed AI with sex-sorted* [in Portuguese]. São Paulo, SP: University of São Paulo. Dissertation.

Norman HD, Hutchison JL, Miller RH. 2010. Use of sexed semen and its effect on conception rate, calf sex, dystocia, and stillbirth of Holsteins in the United States. *J Dairy Sci*, 93:3880-3890.

Peippo J, Vartia K, Kananen-Anttila K, Räty M, Korhonen K, Hurme T, Myllymäki H, Sairanen A, Mäki-Tanila A. 2009. Embryo production from superovulated Holstein-Friesian dairy heifers and cows after insemination with frozen-thawed sex-sorted X spermatozoa or unsorted semen. *Anim Reprod Sci*, 111:80-92.

Perry GA, Smith MF, Lucy MC, Green JA, Parks TE, Macneil MD, Roberts AJ, Geary TW. 2005. Relationship between follicle size at insemination and pregnancy success. *Proc Natl Acad Sci USA*, 102:5268-5273.

Perry GA, Smith MF, Roberts AJ, Macneil MD, Geary TW. 2007. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. *J Anim Sci*, 85:684-689.

Rath D, Barcikowski S, De Graaf S, Garrels W, Grossfeld R, Klein S, Knabe W, Knorr C, Kues W, Meyer H, Michl J, Moench-Tegeder G, Rehbock C, Taylor U, Washausen S. 2013. Sex selection of sperm in farm animals: status report and developmental prospects. *Reproduction*, 145:R15-R30.

Roelofs JB, Graat EaM, Mullaart E, Soede NM, Voskamp-Harkema W, Kemp B. 2006. Effects of insemination-ovulation interval on fertilization rates and embryo characteristics in dairy cattle. *Theriogenology*, 66:2173-2181.

Sá Filho MF, Ayres H, Ferreira RM, Nichi M, Fosado M, Campos Filho EP, Baruselli PS. 2010a. Strategies to improve pregnancy per insemination using sex-sorted semen in dairy heifers detected in estrus. *Theriogenology*, 74:1636-1642.

Sá Filho MF, Crespilho AM, Santos JEP, Perry GA, Baruselli PS. 2010b. Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled *Bos indicus* cows. *Anim Reprod Sci*, 120:23-30.

Sá Filho MF, Santos JEP, Ferreira RM, Sales JNS, Baruselli PS. 2011. Importance of estrus on pregnancy per insemination in suckled *Bos indicus* cows submitted to estradiol/progesterone-based timed insemination protocols. *Theriogenology*, 76:455-463.

Sá Filho MF, Girotto R, Abe EK, Penteado L, Campos Filho EP, Moreno JF, Sala RV, Nichi M, Baruselli PS. 2012. Optimizing the use of sex-sorted sperm in timed artificial insemination programs for suckled beef cows. *J Anim Sci*, 90:1816-1823.

Sá Filho MF, Mendanha MF, Sala RV, Carvalho FJ, Guimarães LHC, Baruselli PS. 2013. Use of sex-sorted sperm in lactating dairy cows upon estrus detection or following timed artificial insemination. *Anim Reprod Sci*, 143:19-23.

Santos JEP, Cerri RLA, Sartori R. 2008. Nutritional management of the donor cow. *Theriogenology*, 69:88-97.

Sales JNS, Neves KaL, Souza AH, Crepaldi GA, Sala RV, Fosado M, Campos Filho EP, Faria M, Sá Filho MF, Baruselli PS. 2011. Timing of insemination and fertility in dairy and beef cattle receiving timed artificial insemination using sex-sorted sperm. *Theriogenology*, 76:427-435.

Sartori R, Souza AH, Guenther JN, Caraviello DZ, Geiger, LN, Schenk JL, Wiltbank MC. 2004. Fertilization rate and embryo quality in superovulated Holstein heifers artificially inseminated with X-sorted or unsorted sperm. *Anim Reprod*, 1:86-90.

Sartori R, Bastos MR, Wiltbank MC. 2009. Factors affecting fertilisation and early embryo quality in single- and superovulated dairy cattle. *Reprod Fertil Dev*, 22:151-158.

Schenk JL, Suh TK, Seidel GE Jr. 2006. Embryo production from superovulated cattle following insemination of sexed sperm. *Theriogenology*, 65:299-307.

Schenk JL, Cran DG, Everett RW, Seidel GE Jr. 2009. Pregnancy rates in heifers and cows with cryopreserved sexed sperm: effects of sperm numbers per inseminate, sorting pressure and sperm storage before sorting. *Theriogenology*, 71:717-728.

Seidel GE Jr, Schenk JL, Herickhoff LA, Doyle SP, Brink Z, Green RD, Cran DG. 1999. Insemination of heifers with sexed sperm. *Theriogenology*, 52:1407-1420.

Seidel GE Jr. 2007. Overview of sexing sperm. *Theriogenology*, 68:443-446.

Seidel GE Jr, Schenk JL. 2008. Pregnancy rates in cattle with cryopreserved sexed sperm: effects of sperm numbers per inseminate and site of sperm deposition. *Anim Reprod Sci*, 105:129-138.

Seidel GE Jr. 2014. Update on sexed semen technology in cattle. *Animal*, 8(suppl. 1):160-164.

Soares JG, Martins CM, Carvalho NaT, Nicacio AC, Abreu-Silva AL, Campos Filho EP, Torres Júnior JRS, Sá Filho MF, Baruselli PS. 2011. Timing of insemination using sex-sorted sperm in embryo production with *Bos indicus* and *Bos taurus* superovulated donors. *Anim Reprod Sci*, 127:148-153.

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

Stewart BM, Block J, Morelli P, Navarette AE, Amstalden M, Bonilla L, Hansen PJ, Bilby TR. 2011. Efficacy of embryo transfer in lactating dairy cows during summer using fresh or vitrified embryos produced in vitro with sex-sorted semen. *J Dairy Sci*, 94:3437-3445.

Taylor STCS, Moore AJ, Thiessen RB. 1985. Food efficiency in traditional and sex controlled systems of beef production. *Anim Prod*, 40:401-440.

Thomas JM, Lock SL, Poock SE, Ellersieck MR, Smith MF, Patterson DJ. 2014. Delayed insemination of non-estrous cows improves pregnancy rates when using sex-sorted semen in timed artificial insemination of suckled beef cows. *J Anim Sci*, 92:1747-1752.