SEXED SPERM VS CONVENTIONAL SPERM – A COMPARATIVE DISCUSSION

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Introduction

An estimate of male reproductive function in any of the productive animal species is a topic of varied interpretation. In its simplest form, the aspect of natural mating provides a direct estimate of the fertility potential of a given male. In cattle, in a herd situation, if a male is allowed to naturally service a number of females over a sixty to ninety day period, the fertility potential of the male is the percentage of females successfully fertilised and pregnant. However, this is not easily correlated with the widespread use of this male for artificial breeding. Many other factors come into play such as the quality of the individual ejaculate, the variation between ejaculates, dilution of semen, sperm dose rate per breeding unit, the functional competence of this sperm, the inherent fertility of the females inseminated with this semen, management factors within the herd environment and so on (Amann, 1989, Amann and DeJarnette, 2012). When you overlay sperm sexing into this mix, the evaluation of male fertility becomes even more complicated. A comparison between conventional or unsorted sperm and sex-sorted sperm is at the mercy of many variables that play a minute to a rather significant part in influencing the outcome. It will be useful to describe some of the major differences between these two processing methods and what effect it has on the outcome (pregnancy rates).

Differences in semen processing Conventional vs Sex-sorted sperm

A high level overview will provide an appreciation of the differences between the two types of processing. While conventional semen processing has minimal intervention points, about three or four depending on the processing method, sex sorted semen on the other had goes through over 21 process steps before it is subject to cryopreservation (Vishwanath, 2013). Each step is physically and bio-chemically demanding on the sperm cell and the logical conclusion is that this sperm cell should or will be compromised in terms of its function. Andrologists over the years have used a rather blunt tool in increasing sperm concentration to accommodate the adverse effects of compromised sperm (Pace et al 1981, den Daas et al, 1998). In some cases this strategy has worked. This will be discussed in more detail in subsequent sections.

Fertility of Conventional and Sex sorted sperm

The field fertility of sex sorted sperm has been discussed in many reviews and the references therein (Seidel 2012, Seidel and Garner, 2002, de Graaf et al, 2014). In all these publications the most quoted issue is the relative fertility of sex-sorted sperm compared with unsorted sperm. It has been an axiom that sex sorted semen in cattle has always lagged behind conventional semen in terms of fertility. The compensable elements that normally would lift the sub fertile individual into one with average fertility such as higher sperm numbers or a higher proportion of sperm with better morphological features have not yielded better results with sexed sperm (De Jarnette et al 2011). The economics of this technology and its application in both cattle breeding as well as herd improvement is well understood, yet, the prevailing opinion is that this less than

optimum fertility has been the reason why this technology has not been widely adopted (Seidel 2014).

The difference in fertility between conventional semen and sex-sorted semen is considered to be in the order of 10 percentage points, and this gap is not bridged by increasing the number of sex sorted sperm per inseminate (DeJarnette et al 2011). The causes for this difference in fertility have been attributed to the varied biochemical changes that sperm undergo during the process of sex sorting. There are over 21 different sub-processes involved in sex sorting which includes an extended holding time prior to staining, exposure to a laser beam to fluoresce and be discriminated into X and Y sperm and finally exposure to an electrical field for drafting as a pure population into an appropriate vessel, All of this in some part is believed to contribute to this fertility difference (Seidel and Garner 2002). The challenge therefore has been to seek imaginative ways in improving sex sorting through new hardware and software as well as new semen processing techniques during the pre and post sorting phases.

This brings about a new point of discussion. Where exactly is the main lesion that actually causes this drop in fertility with sex-sorted semen?

Sperm Heterogeneity – a factor that affects in vivo fertility of sex sorted semen

It is important to define the term sperm heterogeneity and over the decades it has been recognized with some quite distinct functions. The first is structural heterogeneity between sperm within a sample. As early as 1973, Bedford et al described quite elegantly the variations in human sperm nuclear chromatin assembly with some distinct pattern and arrangements (Bedford, 1973). Similar such observations were seen in bull sperm with a comment on association of such variation with fertility (Ballachey et al 1987).

The second is functional heterogeneity and this has been explained by classical competition studies where adaptation, sexual selection and choice of mate due to fitness traits is done through physiological means by the female reproductive tract (Curtsinger, 1991, Birkhead and Moller, 1993, Birkhead and Pizzari, 2002). Several studies have tried to rationalize these concepts through some innovative experiments such as heterospermic inseminations (Beatty et al 1969). These concepts have been described in more detail with an accompanying thesis on how to exploit this heterogeneity to develop rational laboratory tests for sperm competence (Holt and van Look, 2004).

The third concept is physiological heterogeneity between populations of sperm within an ejaculate. The simple explanation for this is where a semen sample has distinct sub populations that would physiologically be ready for fertilisation at different times post insemination. This will allow some flexibility from the time the sperm enter the female reproductive tract to the time when ovulation occurs and a competent sub set of sperm are available and ready for fertilization. The variation in fertility of a given semen sample or of semen samples from the same individual is attributed to this diversity in a sperm population within an ejaculate (Rodriguez- Martinez 2006). If this heterogeneity in sperm population is disturbed, it is more than likely to lead to sub fertility or infertility. A good illustration of this concept can be seen in trials with fresh encapsulated sperm compared with fresh conventional sperm (McMillan and Vishwanath 1994, Vishwanath et al 1997).

The effect of sperm numbers and cryopreservation – sexed sperm and conventional

The relationship between sperm number and the fertility of a given semen sample is well understood. Studies by Pace et al 1981 and Den Daas et al 1998 showed that increasing sperm numbers increased fertility until it reached the asymptotic maximum for the given bull. While individual bulls varied in their absolute fertility, once they reached their asymptote, increasing sperm numbers did not alter this maximum. It is reasonable to assume that this concept would hold true with sex-sorted sperm as well. This theory was tested in one study where comparable numbers of sex-sorted and control sperm still showed decreased fertility (Frijters et al 2009) and in a separate study where increasing the number of sex sorted sperm from 2.1 million to 10 million per inseminate also did not improve relative fertility compared with control non sorted sperm (Table-1, De Jarnette et al 2011). It is plausible that sex sorted sperm are physiologically different compared with unsorted sperm and the usual compensable elements such as increasing sperm numbers improving in vivo fertility does not apply in this case. It is important to note that sex sorted sperm has never been tested at extremely high concentrations such as 10 to 25 million per inseminate. It is neither practical nor economical to do so given the constraints of the sex sorting process.

Table 1. Conception rates and relative fertility of sex sorted semen at 2.1 million and 10 million sperm per insemination. Data from DeJarnette et al 2011

Sex sorted		Conventional		
Sperm concentration	Conception rate	Sperm concentration	Conception rate	Relative fertility
2.1×10^6	38%	2.1×10^6	55%	70%
10×10^6	44%	10×10^6	60%	73%

There is good evidence that cryopreservation in itself causes irreparable damage to the inherent fertility potential of conventional sperm. The important factor here is that the heterogeneous sperm populations in conventional semen samples react differently to the cryopreservation process and hence the detrimental effect of cryopreservation is countered by increasing sperm numbers. Studies with optimal and sub optimal numbers of sperm used as unfrozen or cryopreserved semen show vastly different responses in overall fertility. There is a clear bull x dose rate interaction when conventional semen is cryopreserved and hence a five-fold increase in sperm numbers is required to fully compensate for cryopreserved damage compared with unfrozen semen (Shannon and Vishwanath 1995).

Lessons from Fresh Sex Sorted Sperm – New Zealand trials

The interaction between the process of sex sorting and subsequent cryopreservation is possibly multiplicative (Seidel 2012). Therefore a lot more of sex-sorted sperm are required to reach full fertility. This is in fact true and there is growing evidence that the actual process of sex sorting itself is not quite as damaging and fertility with fresh sex sorted semen is only slightly less than that of unsorted semen. Large-scale fertility trials over the last three years in New Zealand provide good evidence that sex sorted sperm at a concentration of 1 million has a relative fertility of around 95% to that of conventional sperm at a concentration of 2 million (Tables – 2 and 3).

Table 2. Field evaluation of fresh sex sorted semen in New Zealand. 18 – 24 day NRR of fresh sex sorted (1 million) or conventional semen (2 million).

Season	Sex sorted		Conventional		SS – Conv	SS / Conv
	Insems	NRR %	Insems	NRR %	NRR %	%
2011	8,848	69.4	10,981	73.6	-4.2	94.3
2012	18,760	68.1	19,915	72.3	-4.2	94.2
2013	26,104	69.9	26,189	73.4	-3.6	95.1
Total	51,712	69.1	57,085	73.1	-3.9	94.6

Data from Z Xu 2014, LIC, New Zealand. In press J Dairy Science
Results are 18-24 day NRR
All inseminations in lactating dairy cows

Table 3. Field evaluation data, New Zealand. Calving statistics with fresh sex sorted or conventional semen

	2011			2012		
	Sex Sorted	Conventional	SS-Conv	Sex Sorted	Conventional	SS-Conv
No of AI	14,239	17,372		31,051	31,294	
Calving / AI %	51.2	54.3	-3.1	49.7	52.6	-3

Data from Z Xu, J Dairy Sci in press
Calving / AI %, is adjusted calving taking into account AI in culled cows and AI in non pregnant cows

New developments in sex sorting and processing: Trials with SexedULTRA™

Over the last few years' new methods have been developed in semen handling and processing before, during and after the sorting process (Lenz et al, 2014, deGraf et al 2014). Simultaneously, there has been remarkable progress in sperm sorter technology with the new generation Genesis sorters. These machines have enhanced digital electronics with considerable automation and a much smaller footprint (Sharpe and Evans 2009, Evans 2010, Vishwanath et al, 2014). These multiple changes to the sex sorting process have led to a new product called SexedULTRA™. This product went through extensive in vitro lab trials as well as many small-scale field fertility trials over the last 2 years. In vitro lab trials showed promising improvements in all sperm parameters tested (Figure 1).

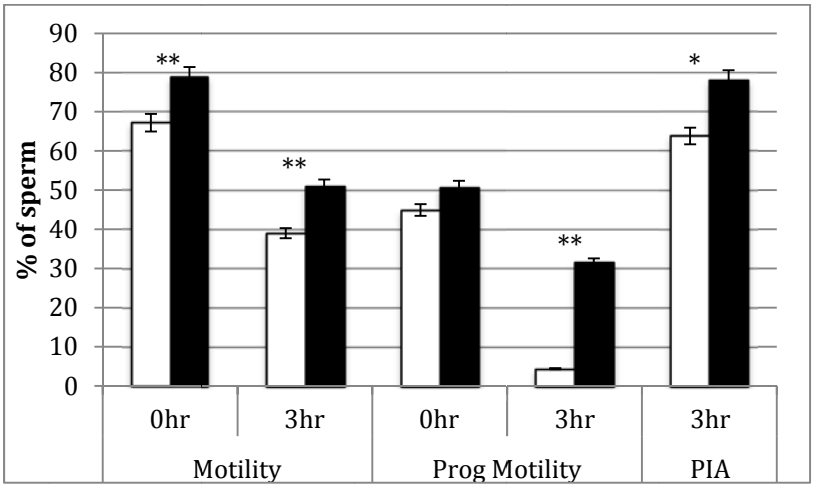

Figure 1. SexedULTRA™ method improves in vitro sperm characteristics compared with the XY method. (Lenz et al, 2014)

A balanced field fertility evaluation of SexedULTRA™ was then conducted in collaboration with Select Sires. Semen from 8 Holstein bulls were submitted to SexedULTRA™ or XY processing methods and used to inseminate 6,930 Holstein heifers across 41 commercial herds in the USA. The SexedULTRA™ method resulted in a greater ($P < 0.001$) conception rate compared to the XY method (45.7 vs. 41.2 %, respectively). This is the first report in many years to show an improvement in fertility of sex-sorted semen (Table-4).

Table 4. Fertility of sex-sorted, frozen-thawed bull spermatozoa processed using traditional XY protocols or the new SexedULTRA™ method and inseminated into Holstein dairy heifers. Scanned pregnancy data from Lenz *et al.* (2014).

Method of processing sexed bull spermatozoa	Number of inseminations	Scanned pregnancy rate
XY	3384	41.6%[a]
SexedUltra™	3546	46.1%[b]

Values without common superscripts differ significantly ($P<0.01$)

These first field fertility trials were then followed up with a further enhancement to the SexedULTRA™ product and tested in a dose rate trial with German Genetics International (GGI) in Germany as the industry partner. Results from over 6,000 trial inseminations from five bulls in over 50 herds show for the first time an improvement in conception rate with increasing sperm concentration. Also for the first time, no detectable difference in conception rate was noticed with 4 million sex sorted sperm compared with 15 million conventional sperm (Table-5). The result is very encouraging and points to a significant improvement in processing and sorting technology and consequently conception rates with sex sorted sperm.

Table 5. Effect of increasing dose rates of sex sorted semen on field fertility. Sex sorted semen processed as the new SexedULTRA compared with XY method at 2.1 million and Conventional (15 million). Data produced in collaboration with GGI, Germany.

Treatment	**Number of inseminations**	**56 day NRR (%)**	**Relative Fertility**
XY method	1953	55.9[A]	84%
SU 2.1 mill	1999	59.9[B]	90%
SU 3 mill	2013	60.0[B]	90%
SU 4 mill	1890	66.7[C]	100%
Conv (15 mill)	62398	66.5[C]	

Data from cows and heifers. NRR results with different superscripts are significantly different $P < 0.05$

Future goals

It is now clear that the gap in fertility between conventional and sex sorted sperm is declining rapidly. Anecdotal reports suggest equivalent fertility of sex sorted and conventional sperm in well managed herds where there is great attention to herd health and reproductive management

(Hippen, Lundgren and Lenz, 2015, personal communication). Similar reports are now coming in from Veterinary practices in Australia where the use of sex sorted semen in timed inseminations on synchronised dairy animals show equivalent fertility to that of conventional semen. This has been reported for both heifers and cows (Jon Kelly, Warnambool Vet Clinic, Victoria, Australia, TLG meeting, Noorat Victoria).

The audacious goal now is to see if sex sorted sperm during certain circumstances, are more fertile than conventional semen. This is not as utopian as it sounds. There is evidence that sex sorted sheep sperm are distinctly more fertile than their conventional counterparts (de Graf et al 2014). The overall interaction of heterogeneity, cryopreservation and timing of insemination needs to be investigated to further refine this sexed semen technology.

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