



Sexed Semen and Major Factors Affecting Its Conception Rate in Dairy Cattle

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Abstract

Sexed semen is preferable by dairy farmers for sustainability of milk production, optimizing the number of herds and weight gain for beef purposes and use of sex-sorted semen facilitates more rapid genetic progress through increased selection intensity. However, adoption of sexed semen for AI in heifers and cows has been limited by cost, low conception rates, and sexing accuracy. Among the major factors that affect the adoption of sexed semen, conception rates were a great problem in the success of AI sexed semen. Conception rates are reduced 10-30% in sexed semen as compared to conventional semen due to many factors. Relatively conception rates in cows are lower than that of heifers because of postpartum stress in cows. For these reason, sexed semen is usually not profitable in dairy cows, unless the fertility is almost equal to conventional semen. If the genetic merit of animals is known, sexed semen could be profitable on genetically better animals while it is not profitable on genetically poorer animals. Beside the economy, high cost of sorting machine and sperm per straw, unavailability of improved bull and poor importing system are major challenges on the application and feasibility of this technology in the developing countries.

Keywords: Conception rate, Flow Cytometry, Sexed semen, Dairy cattle

Introduction

Reproductive technologies such as artificial insemination (AI), multiple ovulation and embryo transfer(MOET), and semen sexing are effective tools in achieving greater genetic improvement in dairy cattle relative to the conventional natural mating systems. Artificial insemination has probably been the greatest technological advance in animal breeding. The main reasons for its success have been genetic gain, disease control, and the cost-effectiveness of insemination compared with natural mating (Vishwanath, 2003).The latest breakthrough in animal reproduction is sexing of semen. Sperm sexing is an assisted reproductive technology that can increase the

efficiency of breeding programs in dairy herds. Sex preselection is more advantageous for productive purposes than embryo sexing. Hundreds of thousands of calves have been born from sexed sperm (SS). Most of these calves were produced in the USA, UK, Argentina, Brazil, and Mexico, with lesser numbers in several other countries. Currently, sexed bovine sperm can be purchased from companies in the UK, Canada, USA, Mexico, Argentina, Brazil and China. In several countries, the licensing and commercialization of this biotechnology is in various phases of development(Garner, 2006). Companies have to decide exactly what products might be provided (e.g., fresh and/or frozen sperm, number of sperms/dose, which class of bull(Seidel J, 2003).

Accuracy of the process is about 90% for either sex, and the resulting calves appear to be no different from non-sexed controls in birth weight, mortality, rate of body weight gain, and incidence of abnormalities (Seidel J, 2003). Sorted sperm can also be used for in vitro production of embryos and large-scale production is on the way to practical application (Wheeler *et al.*, 2006). Encouraging results have been obtained in China, where healthy offspring was achieved with sexed IVF embryos. Sexed sperm has been also used in ICSI procedures where normal calves have been obtained (Hamano *et al.*, 1999). Although SS tends to degenerate faster than normal sperm, cryopreservation overcomes this problem allowing its use anywhere in the world. Pregnancies have been reported with sperm that was frozen-thawed before being sorted and refrozen thawed after sexing (Underwood *et al.*, 2010). Using beef semen in dairy herds allows farmers to produce crossbred calves whose carcasses are more valuable than those of purebred calves (Wolfová *et al.*, 2007). Furthermore, the genetic level of the herd may increase when beef semen is used in genetically inferior cows, because the offspring of these cows do not enter the milking herd (Ettema *et al.*, 2017). In farm animals, especially cattle, there are economic requests of farmers that the offspring are desired to be of a particular sex. Specifically, dairy farmers are engaged in the production of milk, thus preferring female calves and tend to consider male calves as byproducts (Naniwa *et al.*, 2019). The main goal in the use of sexed semen is to produce calves of a specific sex (Razmkabir, 2018).

Even though, sexed semen have several advantages, reduction in the number of cows needed for progeny testing, the opportunity of using fewer and genetically better cows for producing replacement dairy heifers and optimize the herds and a wider opportunity for crossbreeding of dairy cows with both dairy or beef bulls, decrease of calving difficulty in primiparous heifers (George S, 2003) and in beef cattle, production of meat and weight gain are the main traits (Dominguez *et al.*, 2011). There are a lot of factors that affect the success of sexed semen in dairy cattle. Since there is and poor information exists about sexed semen and factors influencing its conception rate in dairy cattle.

Therefore, the objectives of this review were, to indicate the application, to identify major factors influencing efficiency and to insight constraints and challenges of sexed semen in cattle production.

Application of sexed semen

By far, the main application of sexed semen is to increase the percentage of heifer calves to expand the herd or sell replacements. There are two main reasons for wanting more heifer calves: herd expansion and sale of heifers to others. At any given time, a fair number of dairy herds are expanding, and there are several important reasons for expanding from within the herd. The main reason over the years has been to build on known genetics. Apart from improving the genetic potential it also helps in preventing the transmission of some venereal infectious diseases, like vibriosis and trichomoniasis, brucellosis, tuberculosis and IBR that are usually spread through natural mating (Sharma *et al.*, 2018).

Additionally, breeding heifers to have heifer calves to decrease the incidence of calving difficulty, a major problem is dystocia when heifers have their first calf. This can be minimized by breeding only well grown (but not fat) heifers and by using service sires that produce a low percentage of difficult births. A large study in New Zealand with primiparous beef heifers Morris *et al.*, (1992) illustrates sex differences well; death losses from birth to weaning were 10% for heifer calves and 18% for bull calves, mostly due to sequelae of dystocia. To decrease dystocia substantially, one could use bulls that sire a low percentage of calves with difficult births plus semen sexed to produce 90% heifer calves. There is the added benefit that these first calf heifers should be better genetically, on average, than the older cows in the herd, so the resulting calves should be excellent replacements. The application of sexed semen is useful in IVF. The first calves produced with accurately sexed semen resulted from in vitro fertilization (IVF), which requires fewer sperm than artificial insemination were reported (Cran, 1993).

Accuracy of sexing sperm was 90%. With IVF or superovulation, one might want bull or heifer calves, depending which sex is most valuable. Fortunately, several companies provide IVF services using sexed sperm. This is more successful for semen from some bulls than others. In-vitro fertilization reduces the number of sorted sperm needed to fertilize an oocyte. Artificial Insemination or Multiple Ovulation Embryo Transfer requires millions of sperm cells for successful fertilization. In vitro fertilization requires only about 600-1500 sorted sperm cells to fertilize an oocyte (Hall, 2011).

Factors affecting the conception rate of sexed semen

Sorting process

The decreased conception rates in sexed semen are attributed to the stress associated with the sorting process. The stress put on sperm cells includes the diluting of the semen sample, dyeing the sperm cells with a DNA binding agent (Hoechst 33342), mechanical forces including being sent through the flow cytometer at 60 miles per hour at 40 pounds per square inch (De Vries and Nebel, 2009) light from the laser used to illuminate the DNA, pressure from the collection process, and finally, centrifugation to purify the sample (Cerchiaro *et al.*, 2007). Sex-sorted semen does not survive cryopreservation as well as conventional semen (Garner and Seidel J, 2003).

Reduced fertility, when using sorted sperm, has been attributed to the damage of spermatozoa caused by the sexing process (Seidel and Garner, 2002). This includes staining and incubation of spermatozoa with Hoechst 33342, sperm dilution, exposure to high pressure and laser light, rapid projection into the collection tube, and centrifugation to concentrate sorted sperm. After sorting, spermatozoa are partially capacitated resulting in a shorter life span and consequently in reduced fertilizing capacity (Vazquez *et al.*, 2003). Additionally the nature of the damage to sperm has not been explained adequately by research, but there are many possibilities, including stretching the sperm tail as droplets are formed at the nozzle opening, and continued binding of Hoechst 33342 to sperm post fertilization, slowing down progression of the first cell cycle between fertilization and the first cleavage (Seidel *et al.*, 2012). An unsettling point is that the damage appears to be only partially compensable by increasing sperm numbers per dose (Seidel, 2014). The technology of semen cell sorting is continuously being improved but the viability and quality of sperm during sorting procedure will be reduced (Razmkabir, 2018).

Number of sperm deposited

Conception rates in sex-sorted semen are also affected by the number of sperm cells per straw. The standard dose for a straw of sexed semen is approximately 2×10^6 sperm cells (Garner and Seidel, 2008; Healy, House and Thomson, 2013). Conventional straws have approximately $15\text{--}20 \times 10^6$ sperm cells per standard dose (Healy *et al.*, 2013). The lower number of sperm

cells in sexed straws is because of the cost of the equipment and expertise required for the sorting process, the time needed to create a dose of sexed semen, and the variability in bulls' semen viability to survive the sorting process.

According to DeJarnette *et al.*, (2009), sex-sorted semen can enhance the differences in sire fertility rates. The reduced number of sperm cells in a dose exposes a sire's fertility which can be easily missed when more sperm cells are present. DeJarnette *et al.*, 2010 found that by increasing the number of sexed semen cells from 2.1×10^6 to 3.5×10^6 did not increase conception rates. Both dosages of sex-sorted semen had conception rates that were approximately 75% of conventional semen. When semen doses were doubled or tripled (4×10^6 or 6×10^6), pregnancy rates only increased slightly (5-7%). Increasing the number of sexed sperm cells present does not compensate for the damage that occurs during the sorting process (Hall, 2011). Seidel Jr *et al.*, 1997 established the concept of low dose insemination with sexed sperm. They obtained a 22.4% calving rate in heifers that were inseminated with $1\text{--}2 \times 10^5$ non-frozen, sexed sperm. Soon thereafter, numerous field trials were conducted with fewer numbers of nonfrozen and frozen/thawed sexed sperm. For most bulls in these field trials, pregnancy rates with $1\text{--}1.5 \times 10^6$ sexed frozen/thawed sperm inseminated into either the uterine horns or body were 70-90% of unsexed controls containing $20\text{--}40 \times 10^6$ frozen/thawed sperm inseminated conventionally.

Site of semen deposition

During mating, the bull deposits several billions of spermatozoa into the anterior vagina. However, because the cervix is a major obstacle for sperm transport, the number of spermatozoa that finally reach the uterine body usually does not exceed 1% (Harper, 1982). In artificial insemination, semen is generally deposited directly into the uterine body, thus bypassing the cervix and permitting the use of a considerably reduced number of sperm (López-Gatius, 2000).

Many studies have compared conventional semen deposition near the greater curvature of the uterine horns with deposition in the uterine body. (López-Gatius, 2000; Williams *et al.*, 1988) and (McKenna *et al.*, 1990) found no difference in fertility when comparing uterine body and uterine horn insemination. Furthermore, (Diskin *et al.*, 2004) reported an inseminator and site of conventional semen deposition

effect (interaction), with evidence of either an increase, decrease, or no effect of uterine horn deposition on P/AI for individual inseminators. In a competitive insemination study, (Dalton *et al.*, 1999) reported a slight advantage in accessory sperm number attributed to conventional semen deposition near the utero-tubal junction compared with deposition in the uterine body. In Nellore cows, (Meirelles *et al.*, 2012), using conventional semen, reported increased fertility following deep intrauterine AI in the horn ipsilateral to the dominant follicle, as compared to seminal deposition in the uterine body. In contrast, (Carvalho *et al.*, 2012) reported that deposition of conventional semen in the uterine horns failed to improve fertilization rates in super ovulated Holstein cows. Because of the lower numbers of sperm used in sexed semen, it is reasonable to hypothesize that placement of the semen within the reproduction tract would be critical and that deep intrauterine horn insemination would lead to higher CRs. However, Seidel, (1999); and Seidel J and Schenk, (2008) found no evidence that deposition of sexed semen in the uterine horns was superior to uterine body deposition.

Confirmation of estrus and inseminator skills

Man is no match for a bull at detecting estrus in the cow. Incorrect estrus detection is the most common and expensive cause of failure of AI programs. Cows are often falsely identified as being in estrus and inseminated when conception cannot occur (Roelofs *et al.*, 2010). Inseminating the cow is the final, but by no means the least important, step in the process of estrus detection. Although professional inseminators palpate the reproductive tract of numerous cows every day, most are not trained to examine the uterus and ovaries and therefore to confirm estrus. This poses a serious practical limitation to the success of estrus detection procedures and AI. Multiple AIs are undertaken in cows that are not ready for service or are pregnant (Roelofs *et al.*, 2010). The situation is further worsened by the fact that the insemination of pregnant cows can cause embryonic mortality or abortion. The occurrence of estrus during pregnancy has been extensively reported. Pregnant cows stood willingly to be mounted by another cow or bull at all stages of pregnancy (Thomas and Dobson, 1989) and more than 40% of cows with high milk progesterone levels may be inseminated (Nebel *et al.*, 1987). Thus, the first goal of estrus confirmation be to positively identify estrus and to reject cows for insemination that are not ready for service or are pregnant.

Rectal examination of the bovine reproductive tract either by hand or by ultrasonography allows for a correct diagnosis of estrus when the animal is ready for service (Roelofs *et al.*, 2010). Careful examination of the reproductive tract does not seem to impair uterine or ovarian physiology. A cow can be classified as ready for service when the corpus luteum is manually or by ultrasound estimated to be either less than 10mm or non-detectable, the largest follicle shows some fluctuation upon slight pressure and has an estimated diameter of 12 to 25 mm, the uterus is highly turgid and contractile to the touch, and vaginal discharges are copious, fluid, and transparent (López-Gatius and Camóñ-Urgel, 1991). Vaginal fluid can easily be obtained at the time of insemination by gentle suction from the cranial vagina using a plastic inseminating sheath and a 50-mL syringe and examined for transparency, fluidity, and blood or pus contents (López-Gatius *et al.*, 1996).

In a study of estrus confirmation by transrectal palpation performed on 6084 normal repeat dairy cows following the first AI (López-Gatius and Camóñ-Urgel, 1991) the 150-day nonreturn rate for rejected cows with a corpus luteum greater than 15mm in diameter was 87%. Data from a subsequent larger study (Sturman *et al.*, 2000) confirmed the benefit of examining the reproductive tract per rectum at the time of insemination to inseminate cows with estrus only. In Israel, inseminators are trained to check the tone and symmetry of the uterus by transrectal palpation and to examine vaginal fluid. In this country, Israel, it is common practice to evaluate several factors indicative of estrus before insemination to avoid the insemination of pregnant cows, of cows that are not at the appropriate stage of the estrus cycle, or of those with purulent discharge. During the period of this study performed in Israel (1996 –1997), inseminators rejected about 16% of the cows submitted for reinsemination and the 95% accuracy of rejection was 44% in pregnant cows. The main reasons for rejection were the condition of the vaginal fluid and tone of the uterus. In the same study, it was confirmed that the criteria used for submitting cows for reinsemination in areas around New York State were similar to those used in Israel.

Both the diminished efficiency of AI and failure to detect estrus correctly should warrant the re-evaluation of inseminator training to confirm estrus (Roelofs *et al.*, 2010). Examination of vaginal fluid and uterine palpation before insemination have been shown to improve pregnancy rates, reduce abortions, and reduce

the unnecessary use of semen, all of which contribute to the improved reproductive performance and consequent profitability of dairy herds.

Timing AI

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 (Dransfield *et al.*, 1998; Pursley *et al.*, 1998; Hockey *et al.*, 2010). The 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 (Saacke, 2008). 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 (Dransfield *et al.*, 1998). 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 (Maxwell *et al.*, 2004), fewer numbers of sorted sperm/straw (DeJarnette *et al.*, 2008) and possible pre-capacitation induced by the sorting procedure (Lu and Seidel J, 2004). In a small field trial, Schenk *et al.*, (2009) 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.

Thawing procedure

Frozen semen in straw has become the universally accepted unit of storage and transfer of bovine genetics to cattle procedures, which depends on preserving the functional activity of spermatozoa (viability and fertilizing ability) (Bearden *et al.*, 2004). High viability and motility of spermatozoa are important factors for successful artificial insemination (AI) because a significant correlation between post-thawing sperm viability and subsequent conception rate has been reported (Correa *et al.*, 1996). The freezing and thawing of semen inevitably reduces the proportion of motile spermatozoa and causes ultra-

structural, biochemical and functional damages (Senger, 1980). It has been shown that an increase in post-thaw viability will result in increased fertility of the semen (Rastegarnia *et al.*, 2013). Thawing procedure is just as important as the freezing procedure in terms of its impact on the survival of spermatozoa (Nur *et al.*, 2003).

Many researchers have been conducted to determine the optimal thawing temperature duration and increase to know the adequate thawing rate that may give the highest percentage of viable spermatozoa after post-thawing process (Pace *et al.*, 1981; Correa *et al.*, 1996). However, a number of studies have shown that thawing temperatures as high as 60–80 °C could further improve post-thaw motility (Dhami *et al.*, 1996). In some countries, pellets of sperm thawed at +55 °C. Some researchers propose to use for thawing frozen semen of bulls at higher temperatures - from +50 °C to +75 °C or even 100–150 °C (Dhami *et al.*, 1996). Many studies have been conducted to assess the influence of high thawing temperatures on sperm survival and motility, using different thawing rates in bulls (Senger, 1980; Al-Badry, 2012). Al-Badry, (2012) conducted research in Iraq, frozen Semen was thawed in the following procedures: 5 °C for 30 min, 37 °C for 20 sec, 37 °C for 30 sec and 60 °C for 8 sec and motility, live, morphology and Post-thaw viability of sperm cells was assessed by determining the percentage of progressively motile sperm at 0, 2 and 4 h of incubation at 37 °C. Results revealed that motility, live abnormality, intact acrosome, and Post-thaw viability of sperm cells were significantly ($p < 0.05$) higher for 37 °C for 30 sec and 60 °C for 8 sec than the other thawing methods and post-thaw semen can maintained at water bath 37 °C until 3 h. Furthermore, thawing procedure at 37 °C for 30 sec, it is recommended to use in Iraq because it was showing good quality of post-thawing semen and easy to be used in field.

Constraints of sexed semen

The biggest disadvantage of sex-sorted semen is decreased conception rate. Numerous studies have shown conception rates can vary from approximately 60–90% of conventional semen (Loggan, 2019). A straw of sex-sorted sperm is approximately \$15–\$50 more expensive than a conventional straw of semen, depending on the bull. The additional cost for a straw of sexed semen is associated with the high cost of a single flow cytometer, approximately \$340,000 and

the expertise required of individuals working with flow cytometer (Loggan, 2019). In addition to the added cost per straw of sexed semen compared to conventional semen, another disadvantage of sexed semen is the economic impact on the price of female offspring. The supply of dairy replacement heifers will exceed demand. This will reduce the price for replacement heifers. The reduced price for replacement heifers will cause the average price for a cow to decrease. As a result, cull and herd expansion rates are expected to increase. The milk supply will increase, decreasing the price of milk for producers. Moreover, because dairy producers selected for female calves, the price of dairy beef has fallen a result of using heifers in feed lots to produce beef for the meat industry, instead of more feed efficient steer calves (Loggan, 2019). Inbreeding percentages in dairy cattle continue to increase. The continued use of sex-sorted semen will only accelerate the inbreeding percentages because of the limited number of bulls available with sex-sorted semen (Loggan, 2019). Schenk *et al.*, (2006) found in two separate trials, fewer embryos were fertilized when sexed semen was used compared to conventional semen. Sex-sorted semen has further reduced conception rates when utilized alongside fixed-time artificial insemination because females are being inseminated at times not optimal for conception (Thomas *et al.*, 2017). Embryos produced with sex-sorted semen are of lower quality than embryos made with conventional semen (Loggan, 2019).

Challenges in sexed semen

Sexed semen is semen in which the fractions of X-bearing (female) and Y-bearing (male) sperm have been modified from the natural mix through sorting and selection. Sorting is based on flow cytometrical cell sorting for DNA content of sperm (Weigel, 2004; Seidel, 2007). The major challenge of sex sorting procedure is slow and only about 8-10 doses of semen can be sorted each hour. Additionally, the machines are expensive (more than \$300,000 each), so it is a costly process. Because of the costs, there is a higher price put on the semen. Non-sexed semen that would sell for \$15-20 per straw sells \$60 after being sexed. The exposure of sperms to laser light and exposure of droplets to electric charge in machine, reduces the motility of sperms as well as damages the acrosome and membrane (Garner, 2006). Exposure to the dye in combination with the laser may reduce mitochondrial activity in bovine sperms. This results in reduced motility of sperms, because mitochondria produce ATP which is an energy source for sperm

motility (Rai, 2018). The sorted sperm in the sheath fluid is then concentrated by centrifugation. Centrifugation also damages spermatozoa through lipids per oxidation. Due to high dilution in the sheath fluid, the natural antioxidants present in the seminal fluid are lost. The storage of sorted sperms in liquid nitrogen further increases per oxidation of membrane lipids (Rai, 2018).

Conclusion and Recommendations

Sperm sexing is the latest assisted reproductive technology that can increase the efficiency of breeding programs in dairy herds. Sexed semen produced by flow cytometry has the potential to produce offspring of the preferred sex with high accuracy and reliability. Thus, the products are economically beneficial for dairy farmers in terms of obtaining the desired sex in each breed, optimize the herd by heifers' replacement, minimize the risk of introduced diseases and meat gain, and seed stock for beef producers. However, sorting process, bull fertility, improper time and site of insemination, poor confirmation of estrus and inseminator skills detection in cows are the major identified factors that influence the conception rate of sexed semen in dairy cattle. Based up on the above conclusions, the following recommendations are forwarded

- Proper handling technique and exact site of semen deposition is the role factor to achieve pregnancy, in which inseminators have to give attention.
- Insemination of heifers with sex-sorted semen resulted in smaller P/AI, likely because of reduced sperm cells per inseminating dose so that, the number of sperm cells per dose should be greater than that used before.
- Damage to sperm during the sorting process should be managed by replacing new effective and functional machines.
- Cost of sperm per straw and the expensiveness of the sorting machine should be reduced to increase the application and feasibility of the technology through worldwide.
- Extension services must ensure that farmers get adequate information on the input required to benefit from crossbreed dairy cows and from those of higher genetic merit.

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