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Success and Challenges of Sexed Semen in Dairy and Beef Animals: A Review

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Abstract: For many years, humankind has tried to develop methods to select the sex of animals. Sexed sperm can be used to increase the reproductive rates in animals and subsequently increase rates of genetics. Sexed semen is semen which has a modified ratio of X-chromosome (female) bearing sperm to Y-chromosome (male) bearing sperm. Sexed semen contains higher than normal concentrations (up to 90%) of either the 'X' or the 'Y' chromosome. Selection of gender by separating X-bearing sperm from Y-bearing sperm before semen is used in artificial insemination could give farmers the choice of sexed of offspring. Sexed semen is an excellent way to expand the dairy herd without spending large amount on replacement and virtually a breeder need not to buy new heifers. The main benefits of sexed semen were to reduced dystocia, increased genetic gains and heifer rearing, to increase revenues from sale of calves, to produce beef cattle from dairy animals and shortening gestation length, reduced numbers of unwanted male calves and more crossbred calves available for beef sector and increase in the number of dairy heifer calves. The major challenges of sexed semen are the high cost, commercial unavailability of the sorting technology, low sorting speed of efficiency, low conception rate need for standardization, low number of elite bulls, lack of skilled manpower and lack of good quality ejaculates from indigenous cattle. This paper reviewed and discussed the purpose of gender selection, success of sexed semen, time of artificial insemination, semen sexed technology, application of sexed semen in dairy and beef animals, benefits, challenges and of sexed semen. Sexed semen is now routinely used on heifers in many countries practicing year-round calving.

Key words: Challenges · Dairy Animals · Sexed Semen · Success

INTRODUCTION

For many years, humankind has tried to develop methods to select the sex of animals. Various experimental techniques using sperm have been reported, such as density differences [1], weight and electric charge [2], differing protein content [3], immunological properties [4] and chromosome volume [5] among others. About 90% of the new born will be from the required sex [6]. Nowadays, large number of artificial insemination centres offered bovine sexed semen worldwide [7].

Semen sexing is the process of separating spermatozoa into two subpopulations containing X-chromosome and Y-chromosome bearing spermatozoa. The most commonly applied technique for the separation of a fresh semen sample into X and Y-chromosome bearing fractions is the fluorescence activated cell sorting (FACS). Commercialization of X-sorted sexed semen started in 2003 but sales did not take off until early 2006 [8]. The use of sexed semen provided an increase in beef and dairy cattle productive efficiency by the production of an ideal proportion of males and females to obtain advantages from characteristics that are limited or influenced by sex and facilitates economically flexible and practical management [7, 9].

The sexed sperm can be further used to increase the reproductive rates in animals and subsequently increase rates of genetic gain not only from daughter-dam path through possible higher selection intensity and accuracy of selection but also through production of superior male from elite cows for future breeding [7, 10]. Therefore, this review aimed to discuss the success, the challenges and the applications of sexed semen in dairy and beef animals.

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Successes and Challenges of Sexed Semen

Purpose of Gender Pre Selection: Preselecting the gender of offspring in both human and animals has been of keen interest since the beginning of recorded history. Theories for controlling the sex of offspring have been established since the era of the Greek philosophers when Democritus, 470-402 BC, suggested that the right testis produced males, whereas the left testis produced females. Scientific efforts in the right direction started early in the 20th century when first documented microscopic identification of sex chromosomes [11].

Selection of gender by separating X-bearing sperm from Y-bearing sperm before semen is used in artificial insemination could give the farmers of selecting the sex of their offspring [11]. Because the dairy farmer has little use for most bull calves, the use of sexed semen to produce only females would make milk production more efficient. Recently, sex pre-selection before fertilization has been attempted in the field of reproductive biotechnology. The sex of foetuses was determined by whether a sperm, which performs fertilization, carries an X or Y chromosome [12].

Success of Sexed Semen: The majority of studies on sexed sperm have been done with breeding heifers, although some have been done with cows and a few, with superovulation and in vitro fertilization. Importantly and not surprisingly, success varies markedly with management, female age and parity. Because sperm are slightly compromised in the process of sexing and the low number of sperm per dose, everything must be done optimally for good success rates [12]. The following are especially important: Well managed animals, including good nutrition; extremely careful handling of semen including rapid transfer of straws from container to container, thawing in a 95°F water bath and inseminating within 10 minutes of thawing; excellent estrus detection and well trained inseminators [12, 13].

Reproductive Status of Heifers vs. Cows: Fertility in heifers is generally higher than in cows [14]. Therefore some researchers were studying these differences in fertility between the two groups. The author suggested [15] that excellent reproductive management of heifers appearsed to be the key to achieve comparable pregnancy rates between sexed and unsexed inseminations. In order to increase the probability of acceptable conception rates [6] recommend restricting the use of sexed semen to 1st or 2nd service heifers showing estrus, in agreement with [16], who found decreased pregnancy rates in 3rd service heifers compared to 1st and 2nd service heifers. In cows, however, there appeared to be little influence of service number on conception rates using sexed semen and on overall conception. The use of sexed semen in lactating cows, fixed time AI or problem breeders was specifically discouraged [6, 17, 18].

Semen Sexing Technology: Semen sexing is the process of separating spermatozoa into two subpopulations containing X-chromosome and Y-chromosome bearing spermatozoa. The most commonly applied technique for the separation of a fresh semen sample into X and Y chromosome bearing fractions is fluorescence activated cell sorting (FACS) [19].

Application of Sexed Semen: The application of sexed semen extended to different species [7, 20] and it began to be used with other advanced reproductive techniques such as IVF [21] and super ovulation [17]. In addition to cattle, flow cytometric sperm sexing has been applied to sperm from a variety of mammalian species including sheep, rabbits, swine, horses, elk, cats, dolphins [22] humans, non-human primates and dogs [23].

Application of Sexed Semen in Dairy Animals: Sexed semen is an excellent way to expand the dairy herd without spending large amount on replacement and virtually a breeder need not to buy new heifers. In expanding closed dairy herds, application of sexed semen facilitates faster, more profitable expansion of the herds. While in non-expanding dairy herds, sexed semen may be applied for production of replacement heifers for sale or to increase the value of beef output from the dairy herds. Additionally, application of sexed semen could be an effective tool to improve the annual genetic gain [24, 25] through production of replacement heifers only from the superior genetics cows or higher chance to select the best genetic potential as replacement heifers [24, 25].

The recorded sexed semen conception rate differed from herd to herd [6] and from breed to another [26]. However, the cumulative data stated that the fertility of sexed semen was lower than that of unsexed one [27]. About two-thirds of sexed semen fertility reduction was refereed to lower sperm number in the insemination dose (2-4 million) and the other third was referred to the adverse effects of the sorting procedures [28]. The lower fertility limited the application of sexed semen to heifers those have higher predicted fertility [6]. Some recent

reports recorded similar pregnancy rate after using sexed and non-sexed semen in Holstein heifers [29], non-suckling Nelore cows [27]. Several large scale studies with use of sexed semen in dairy heifers indicated that pregnancy rates were 10% to 20% lower with sexed semen compared to conventional semen [6].

Applications of Sexed Semen in Beef Animals: Gender selected or sexed semen has been commercially available to the dairy industry for almost a decade. However, sexed semen from beef bulls has recently became commercially available. The availability of sexed semen from beef bulls along with concerns about success of the technology at the ranch level has limited the use of sexed semen in purebred and commercial beef operations. Recent changes in semen availability combined with current studies with sexed beef semen were providing insights to the uses, limitations, opportunities and challenges of this technology. Increased sorting capacity allowed the number of beef bulls with gender selected semen available to increase exponentially over the last five years. For the major US AI studs, the number of beef bulls with gender sorted semen available increased from 0 to 70 from 2008 to 2011 [30]. Controlled studies comparing sexed beef semen to conventional semen were considerably more limited that experiments in dairy cattle. The utility and practicality of applications are dynamic as the usefulness depends on price of sexed semen, percentage sorted sex (75% vs. 90%) and current market environment [6]. Recently, several deleterious traits have been identified in purebred beef cattle. In some cases, a significant percentage of the females in a herd were carriers. Sexed semen coupled with MOET or IVF could help purebred operations rapidly replace carrier females with "clean lines" while maintaining some of the genetic progress of their herd [6].

Benefits of Using Sexed Semen: The benefits of sexed semen can be further amplified by coupling it with assisted reproductive techniques like multiple ovulation embryo transfer technology, In-vitro fertilization and Gamete intra-fallopian transfer and Sperm intra-fallopian transfer. In the beef industry, it is handy to be able to select the gender of calves if the producer wants to keep replacement females from certain cows/bulls, or wants just steers from a terminal cross [31].

When the market becomes good for replacement females, breeders may need more heifers and when the market becomes better for steers they may choose to produce mostly male calves. Seedstock producers may select bloodlines for maternal qualities for brood cows and others they were hoping to use for marketing bulls. This technology gives the producers the choice and also enables them to develop an early strategy for a potential future market [6]. The principal benefit of using sexed semen was increased numbers of heifer calves born, with approximately 90% of successful pregnancies resulting in a heifer calf [31]. The subsequent increased availability of replacement heifers may be utilised to expand herd size and production. Alternatively these heifers may be sold as calves, which would increase revenues compared with the sale of lower value dairy bull calves. In seasonal calving systems, the use of male sexed semen from beef sires on later calving cows may also be considered to increase the value of beef output from the dairy herd, as male beef calves traditionally command a premium over females [32].

Conventional artificial insemination methods alone are advantageous to many producers because it allows for a shorter calving season, which creates a more consistent, uniform calf crop. Strong genetics are the backbone to a successful operation year after year, whether it is the dairy industry, seedstock producers or a registered herd operation. Another advantage with artificial insemination is the capability to use sires of superior genetic merit. With a variety of available semen, producers can hand pick which cow/bull combination would create more superior offspring to improve the existing herd [31]. Sexed semen is very beneficial to the dairy industry, because of the high demand for female offspring to serve as replacement females with better milking genetics than the elders. All replacements can be generated on-farm, thereby eliminating the need to buy in stock (and potential exposure to disease) from external sources [33]. The following are some benefits of sexed semen: reduced dystocia, increased genetic gains and heifer rearing, to increase revenues from sale of calves, to produce beef cattle from dairy animals, shortening gestation length and economics use of sexed semen.

Reduced Dystocia: Dystocia impacts the profitability of dairy herds through losses in production, fertility, cow and calf mortality, increased culling and veterinary and management costs. It has been demonstrated that by increasing the proportion of heifer calves born at the expense of heavier male calves, the incidence of dystocia is reduced in 1st-calving heifers [18]. In any given herd, the option of sex determination could be useful for reducing calving difficulty in first calvers (since heifer

calves are typically smaller at birth than bull calves), or for producing a higher number of good females to choose from as replacements if you want to expand your herd size without buying outside cattle [31].

Increased Rate of Genetic Gain: According to [25], the sexed semen may be used to accelerate the rate of genetic gain in dairy herds, by selecting only the highest ranking cows to breed replacements from with sexed semen. Achieving greater genetic gain with sexed semen depends, however, on the availability of sexed semen from the highest genetic merit sires. The demand for conventional semen straws is typically greater than the supply for the highest genetic merit sires. Future improvements in the efficiency of sperm capture during the sorting process would make it feasible and indeed financially attractive, for animal breeding companies to sort semen from high genetic merit sires. When this occurs, herd-owners will be able to breed replacements from only the highest genetic merit dams and sires, allowing selection pressure on both the male and female lines. This would have a favourable effect on the rate of genetic gain in dairy herds.

Heifer Rearing: In order to obtain maximum lifetime milk production, all replacement heifers should be first bred at ~15 months of age (to calve at ~24 months of age) and this is particularly important in seasonal pasture-based systems. An efficient heifer rearing system is essential to meet these targets and ensure that replacement heifers maximize their potential as lactating animals. According to [34] the well grown heifers had greater pubertal rates at mating start date (MSD) and were more profitable over their lifetime due to superior milk production and greater longevity.

Beef Production from the Dairy Herd: In non-expanding herds, the use of sexed semen enables the number of replacement heifers required to maintain herd size to be produced from a smaller proportion of the herd. This provided dairy farmers with the opportunity to increase revenues from the sale of calves for meat production, by breeding the remainder of the herd with semen from beef sires [35]. Suitable beef sires would generate progeny that are easy calving with short gestation length (SGL).

Shortening Gestation Length: One of main drivers of profit produced per individual cow in seasonal-calving systems is lactation length; a cow that calves late in the spring will have a longer dry period (greater cost) and a shorter lactation (reduced income) than a cow that calves at the start of the calving period in late winter/early spring. The relative economic advantage of opting to increase the beef output from the dairy herd or to shorten gestation length will depend on regional differences in milk and beef prices [33].

Economics of Sexed Semen: Most dairy producers have used sexed semen to produce more heifers by using it at every first insemination in virgin heifers that are detected in estrus regardless of the genetic merit of the heifer. Because of high market prices for replacement heifers, high milk prices, reproductive inefficiency of dairy herds and the shortage of heifers for replacements, sexed semen has been used to just get more heifers. Despite of higher price and lower fertility, application of sexed semen had resulted in greater net profit than the unsexed semen at the end of time horizon [36]. The sexed semen conception rate and the extent of sexed semen application within the herd greatly affect the economic impact of sexed semen application. Previously, several authors addressed the economics of the use of sexed semen. Calculations on the economics of use of sexed semen in production of bred heifers are probably the most accurate. Management of yearling heifers bred with conventional or sexed semen was similar with only pregnancy rates and semen cost as primary variables [37]. Estimation of economic cost or benefit of using sexed semen in postpartum cows was highly speculative and dependent on a number of factors including production costs, current AI usage and pregnancy rates to sexed semen, long-term impacts, production environment and marketing advantages/ opportunities [37].

The main biological and economic factors have now described. Economic analyses that have included some (but not all) of the complex interactions suggested that sexed semen was most valuable in virgin heifers and then primarily in the first insemination and with diminishing returns in later inseminations [38, 39]. There is no reliable rule of thumb that can dictate proper use across the variety of herds, cows and economic scenarios possible. There were two primary negative factors that must be accounted for when considering the use of sexed semen. These were: the price premium of sexed semen compared with conventional; and the reduction in fertility performance of sexed semen compared with conventional. Authors has examined the economic effects of sexed semen use in a variety of scenarios, both in year-round [35] and seasonal production systems [40, 42].

According to [35] described the economic advantage of using sexed semen as a function of interactions among three spheres of influence: the market environment, management practices and technological efficiency. The breeding management practices employed in a herd will play a key role in determining the profitability of sexed semen use [26, 31, 43].

Challenges of Sexed Semen: The following are the major challenges of sexed semen:

- High cost: The machines costs around 4 to 5 crore and along with it royalty should be paid for each dose of semen produced.
- Commercial availability of the sorting technology: The technology is not fully commercially available.
- Lower sorting speed and efficiency: The sorting speed of the machine is 6000 sperms per second and if we run it for 24 hours maximum doses (2 X 106 sperms per dose) which can be produced is 259.2. From an ejaculate 30% sperms will be rejected during the sexing process due to non- detecting precisely for difference in DNA content and out of the detected sperm 50% will be Y bearing; so semen doses harvesting from a bull of good genetic merit will be reduced by 70% through this technology [18].
- Low conception rate: Conception rate is 10-20% lower in sorted sexed-semen compared to conventional semen [18, 31]. The sorting stresses include high dilution rate, staining with the dye, mechanical forces, UV laser light beam and higher fluidic pressure during projection into the collection tube and centrifugation.
- Need for standardization: The technology of sexed semen being patented and used in the western countries, the standardization and production caters mainly to the *Bos Taurus* dairy and beef breeds [31].
- Low number of elite bulls: The low number of elite bulls will limit the options for semen sexing in genetically superior bulls.
- Lack of skilled manpower: Experienced and proven AI technicians are required for inseminations. Furthermore, developing such a scheme for tropical developing countries is constrained by small flock size, indiscriminate mating and absence of pedigree and performance recording.
- Lack of good quality ejaculates from indigenous cattle [31, 32].
- The other main problem with this technology is that partly due to fewer sperm per insemination dose, pregnancy rates are about 10 percentage points lower than with unsexed semen and that is from AI 12 to 24 hours after observed estrus; fertility will be even lower using appointment breeding without heat detection. Most applications concern artificial insemination of heifers; use of sexed semen with dairy heifers has become widespread [31, 32] Impact of Using Sexed Semen on Animal Genetic Evaluations.

The genetic evaluation of dairy animals relies on the collection of pedigree information on the population under consideration, as well as trait information on these individuals and their relatives. Accurate parentage assignment is therefore important to reduce the errors that could occur in extensively farmed systems. Depending on the farming systems, errors in assignment of parentage could vary from around 7% in German dairy cattle [44] to around 15% in New Zealand dairy cattle [45]. When the incidence of incorrect parentage assignment exceeds 15%, the breeding scheme will need to adjust for the population structure and the type of errors that could impact on the validity of the scheme [45, 46].

CONCLUSIONS

Dairy producers have used sexed semen primarily in virgin heifers. Rapid increase in the availability of sexed beef semen and continuing improvements in the technology of producing and using sexed semen is creating an opportunity for leaders in beef reproduction and genetics. A big problem is that sexed sperm are not available for most bulls and especially not from the more popular bulls, because genetic companies can sell all the semen such bulls produce without sexing it. Both improvements in the fertility and reduced cost of the product will have significant implications for the future use of sexed semen in all types of dairy production systems. Sexed semen is now routinely used on heifers in many countries practicing year-round calving.

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