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Small ruminant SexedULTRA™ sperm sex-sorting: Status report and recent developments

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ABSTRACT

Flow cytometry sperm sex-sorting based on the relative DNA difference between X- and Y-chromosome bearing populations is an established method that has allowed the production of pre-sexed offspring in a multitude of species and has been a commercial success in cattle production for the past twenty years. Several improvements to the technology and the processing methods have increased the sorting efficiency of ejaculates and the post-thaw quality of sex-sorted sperm, allowing for the fertility gap between conventional (non-sorted) and SexedULTRA™ sex-sorted sperm to be bridged. Small ruminant industries are now progressively testing sex-sorted sperm for application in their specific niches and environments. A review of the key advances and the recent developments in caprine, ovine and cervine sperm sex-sorting technology are described in this publication.

1. Introduction

In 2011, the world's population reached seven billion people and it is predicted that this figure will rise above ten billion by the turn of the century. In the period between 1961 and 2014, the available agricultural land area decreased from 0.371 to 0.195 ha (Ha) per capita [1]. At the same time, the total number of livestock (cattle, buffaloes, pigs, chickens, sheep and goats) increased from 6.72 to 12.9 billion head [2]. The Food and Agriculture Organization of the United Nations predicts that the world's population will grow to almost 10 billion by 2050, and the demand for food and other agricultural products is projected to rise by 50% between 2012 and 2050. In fact, several countries such as Egypt or Japan, will have less than 0.1 Ha per capita by 2050, and only five countries may have more than 1 ha of agricultural land per person (USA, Brazil, Canada, Russia and Australia) [3]. As these increases occur, the competition for land by both man and livestock will severely intensify [4]. Therefore, the sustainability of the world's livestock industry relies on strategies and initiatives to meet the needs of billions of people in a way that is economical, healthy, and environmentally responsible.

The demand for sheep and goat products is increasing considerably worldwide. These small ruminants are easily managed, require a relatively small initial investment, and have a short generation interval, which results in a fast return on investment for farmers. In the era of scarcity of goods and materials, sheep and goats can provide high-quality animal products such as milk, meat and wool, and create a large avenue for farmers and industries.

The captive deer breeding industry has also experienced an important growth worldwide [5–7], with an increased trend toward deer farming, where the main financial profits rely on antler trophies. There has also been an increase in consumer demand for venison, which is a lean meat high in protein and low in fat. The deer industry allows farmers to maximize land for profit and utilize tracts of marginal land, since these animals are adaptable to many different terrains, consume less fodder than cattle, are less damaging to pastures, mature more quickly, and can reproduce for up to 20 years in captivity. Hunting related-expenditures and money collected in taxes also provide an important revenue that can be used for managing and maintaining the land, enhancing the wildlife habitat.

As the human population increases, all animal industries will face a crisis. Therefore, it is important to utilize modern biotechnologies to promote a sustainable production of agricultural resources and reduce animal wastage. Artificial insemination (AI) has been one of the most important biotechnologies to allow a significant growth and sustainability of small ruminant's industries, and it is extensively used in most production systems nowadays. Artificial insemination has accelerated the genetic progress for economically important traits such as milk production and growth,

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as well as resistance to health-related problems such as hoof rot and parasitism, both heritable traits. The use of AI has also improved biosecurity, minimizing exposure to animal diseases, and alleviated transportation costs associated with moving live animals. However, it was not until recently that the introduction of sex-sorted sperm opened a new window to improve the reproductive efficiency of small ruminants even further, maximizing the use of resources, allowing an optimal production of males and females in production systems, and improving herd management.

2. SexedULTRA™ sex-sorted sperm. History and new developments

In 1989, Dr. Larry Johnson published the first report of live rabbit pups produced by flow cytometry sex-sorted sperm [8]. A culmination of a series of investigations that started in the mid-1970s, this publication marked a breakthrough where the sex outcome of a pregnancy could be skewed in either direction [8]. Since this breakthrough, the technology has been slowly adapted to produce sex-selected offspring in cattle [9], pigs [10], sheep [11], horses [12], humans [13], buffaloes [14], elk [15], dolphins [16], dogs [17], cats [18], deer [19] and goats [20]. Only ten years after the aforementioned births of sex-sorted rabbits, the first commercial straw containing 2.1 $\times$ 10^6 cryopreserved sex-sorted bovine sperm was released to the market for standard AI [21].

The sperm sex-sorting technology is based on quantitative flow cytometry, where sperm cells are labelled with the DNA fluorescent dye Hoechst 33342 [22], hydrodynamically aligned in a discontinuous droplet stream and excited with a UV laser beam. Each drop is charged according to the DNA content of the encased spermatozoon and deflected using an electrostatic field according to their charge. Flow cytometry allows identification and selection of individual sperm with sort purity above 90% of the desired sex [23,24].

Flow cytometry has revealed differences in the magnitude of DNA content difference between X- and Y- chromosome bearing sperm on at least 23 mammalian species [25–30]. In bovine sperm, the average difference is 3.8%, for ovine it is 4.1%, and for cervine and caprine it is closer to 4.4%. The higher DNA content difference in small ruminant semen allows for a greater number of sperm from the desired sex to be recovered at the end of the process (Fig. 1).

Several improvements have happened in the sex-sorting technology since that first commercial straw was released, such as the introduction of orienting nozzles, digital processing, multiple headed sorters, and automation, in a new generation of faster and more efficient sperm sorters known as Genesis [31–33]. Significant enhancements in sperm handling, preparation for sorting and media composition have also allowed for sperm quality and conception rates of sex-sorted sperm to reach levels that are comparable with conventional semen [34–37]. The result is a complete overhaul of the conditions under which sperm is processed and sorted, known commercially as SexedULTRA™. Today, this technology has applications in a wide variety of animal industries, and sperm sorting laboratories are operating commercially in more than 25 laboratories in 15 countries, with an estimated annual production of over 10 million straws [38].

3. Ovine sex-sorted sperm

There are an estimated 1000 million sheep in the world and three major management systems, namely extensive production for wool and meat, intensive dairy production, and traditional pastoralism. The current world consumption of sheep meat stands at about 2.5 kg per person annually out of an annual meat consumption of 41.6 kg per person. As sheep production increases, the use of sex-sorted sperm will provide genetic, management and financial benefits for this industry [39].

In 1996, Catt et al. [11] presented the first report using ram sex-sorted sperm, where 85 conventional, 92 female-sorted and 74 male-sorted ram sperm were injected into *in vitro* matured sheep oocytes and placed into the oviducts of 28 oestrous sheep. One

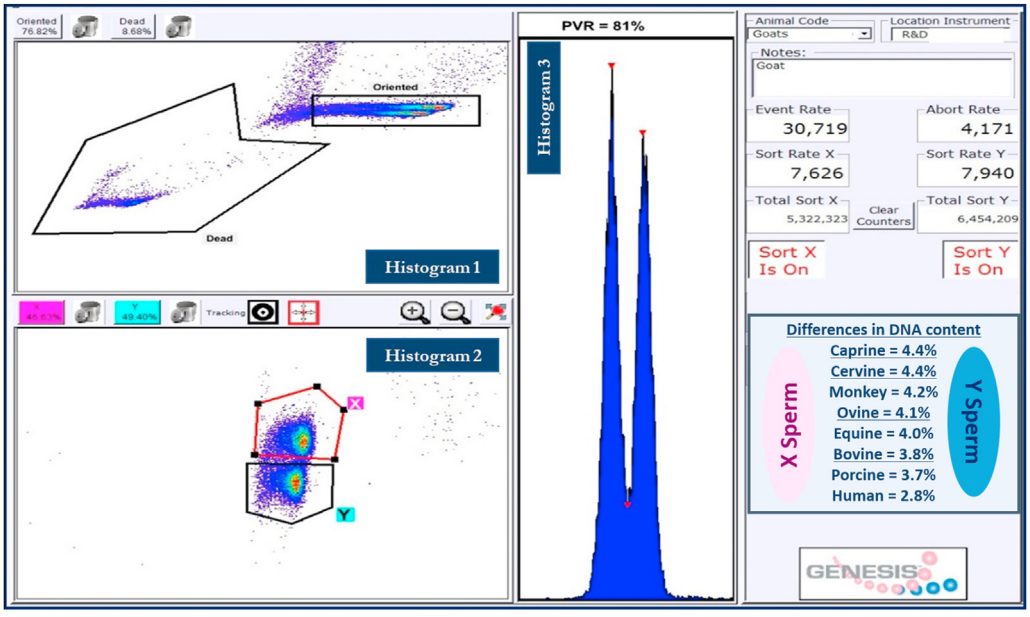

Fig. 1. The populations of sperm are distinguished by histograms on the Genesis flow cytometer software. The first histogram represents Forward 0-degree Fluorescence (FAF) and Side 90-degree Fluorescence (SAF) images to identify live-oriented and dead sperm populations. The live-oriented region is gated into Histogram 2, which is used to analyze and sort on the relative fluorescence of X- and Y- chromosome bearing sperm populations. Histogram 3 monitors resolution by means of peak-to-valley ratio (PVR) percentage, which is a measure of X- and Y- chromosome bearing sperm separation. The difference in DNA content between X- and Y- chromosome bearing sperm varies among species. In bovine sperm, the average difference is 3.8%, for ovine it is 4.1%, and for cervine and caprine it is closer to 4.4%. The higher resolution observed in small ruminant semen allows for a highly efficient and accurate sperm sorting, which results in a higher number of sperm from the desired sex recovered at the end of the process.

pregnancy was diagnosed by ultrasound after 55 days from an oocyte injected with Y- chromosome bearing sperm. Besides this initial study, the use of IVF and ICSI is currently not commercially relevant for the ovine industry, so research has been focused on sex-sorted, fresh and frozen sperm to be used in laparoscopic intrauterine artificial insemination (LAI).

The first pregnancies after LAI with sex-sorted frozen-thawed sperm were achieved using low numbers of sperm per dose (2-4 x 10^6). However, the percentage of ewes pregnant after insemination with either X- (25.0%) or Y- (14.6%) chromosome bearing sex-sorted sperm was significantly lower than for ewes inseminated with conventional controls (54.3%, 140 $\times$ 10^6 sperm) [40]. Further testing of *in vitro* quality parameters of sex-sorted ram spermatozoa showed a reduced total and progressive motility and a tendency towards premature capacitation in sex-sorted sperm compared to conventional [41]. Combining these findings suggested that sex-sorted ram sperm had a reduced fertilizing lifespan, which would explain the decrease in fertility. In subsequent field experiments, the fertility problems were shown to be partly improved by increasing the number of sex-sorted sperm per insemination [41], but this was not a viable solution considering the commercial imperative to minimize the number of sex-sorted sperm per LAI dose.

Only a few years later, de Graaf et al. [42] reported that sex-sorted ram sperm presented higher motility, viability, acrosome integrity and mitochondrial activity than non-sorted controls. *In vivo* studies supported these *in vitro* results, demonstrating that sex-sorted ram sperm resulted in similar or superior lambing percentages than conventional controls when artificially inseminated into superovulated ewes [43], non-superovulated ewes in very low numbers (1 million motile) [44,45], and even when frozen both prior to and following sex-sorting [46]. It appeared that the sex-sorting process could select a functionally superior population of sperm in terms of both *in vitro* and *in vivo* function from the ejaculate, resulting in sex-sorted ram sperm with a superior fertilizing lifespan inside of the female reproductive tract compared with conventional sperm from the same ejaculate. Since that moment, ram sex-sorted sperm was considered an exception to the long-held rule that sex-sorting negatively impacted sperm function to an extent where fertility was compromised [47].

In the past 7 years, researchers at STGenetics® have further improved the sex pre-selection technology to make it a commercially viable and effective reproductive management option for the sheep industry. The research performed has focused on adapting SexedULTRA™ bovine sperm sorting procedures for ovine semen, and to replace the pellet freezing method that was used in all other previous experiments [48] for a straw freezing method, which would make the product a more commercially viable option.

In the first field trial using SexedULTRA™ sex-sorted sheep sperm [49,50], ejaculates from two rams were split and processed in one of two methods: Conventional or sex-sorted. Conventional semen was processed at 60 $\times$ 10^6 per dose and sex-sorted sperm was processed as fresh semen at 1 $\times$ 10^6 and 2 $\times$ 10^6 cells per dose, and cryopreserved in pellets at 3 $\times$ 10^6 cells per dose or in straws at 6 $\times$ 10^6 cells per dose. Sperm motility was greater ($P < 0.05$) in fresh semen compared to cryopreserved semen in pellets at 0 h (73.0 ± 3.0% vs 68.5 ± 3.5%) and after 3 h of incubation at 36 °C incubation (70.5 ± 2.5% vs 63.0 ± 2.0%). Percent total motile sperm was the lowest in cryopreserved straws at 0 h (44.0 ± 7.0%) and 3 h after incubation (43.0 ± 32.0%). The sex purity (X-chromosome bearing sperm) of the sorted sperm was 92.0 ± 0.5%. Estrous synchronization of ewes (n = 285) for this trial was performed using single controlled internal drug releasing (CIDR) devices. On day 10, ewes were injected intramuscularly with 360 IU of pregnant mare's serum gonadotropin (PMSG). CIDR were removed on day 12, and inseminations were performed at 54–56 h after CIDR removal. Ewes that had been inseminated were moved to a separate pen to be housed individually one week prior to the expected parturition date. Fertility rates were assessed based upon delivery rates. Significant differences were not found in the percentage of ewes lambing after insemination between treatments (Table 1), confirming that sex-sorted ram sperm can be equally fertile to conventional. In fact, frozen semen at 6 $\times$ 10^6 cells per dose and fresh semen at 2 $\times$ 10^6 cells per dose presented numerically superior fertility when used in LAI than conventional sperm inseminated at higher concentrations. No differences were observed in fertility between fresh and cryopreserved sex-sorted sperm. However, sorted sperm cryopreserved at 6 $\times$ 10^6 per straw presented numerically higher conception rates than sorted sperm cryopreserved at 3 $\times$ 10^6 pellet, which could indicate a dose rate effect. Previous *in vitro* sperm analyses have demonstrated that the sex-sorting process selects a superior population of sperm from the ejaculates [36,51,52]. The removal of dead and damaged cells through flow cytometric sorting, and the use of the SexedULTRA™ methodology, ensures that sex-sorted sperm are maintained in a benign environment with a balanced pH and low levels of reactive oxygen species, which results in improved sperm characteristics and increased longevity when compared to conventional semen. This improved semen quality could explain the superior fertility of sex-sorted compared with conventional sperm, even when using a lower insemination dose.

In a second field trial to explore dose effects, ejaculates from one ram were split and processed in one of two methods: Conventional or sex-sorted. Conventional semen was processed at 25, 10 and 2 $\times$ 10^6 fresh sperm per dose, and sorted sperm was processed at a sex purity of 90% X-chromosome bearing sperm, at 10 and 2 $\times$ 10^6 fresh sperm per dose. In this trial, ewes (n = 293) were synchronized using CIDRs for 12 days, followed by an intramuscular injection of 360 IU of PMSG at CIDR removal. Ewes were inseminated by laparoscopy at 49–55 h post CIDR removal. Pregnancy status was determined using transcutaneous ultrasound on Day 50–51 post AI. There was not a difference ($P < 0.05$) between the percentage of pregnant ewes after insemination with conventional semen or sex-sorted sperm at 10 $\times$ 10^6 fresh cells per dose, but ewes inseminated with sex-sorted sperm at 2 $\times$ 10^6 fresh sperm per dose recorded a significantly lower pregnancy rate than all other sperm treatment groups (Table 2). This study would also point in the direction of a dose effect, but would contradict the results from the first trial where sex-sorted sperm at 2 $\times$ 10^6 fresh sperm per dose presented superior fertility than conventional sperm inseminated at higher concentrations. This would point out the importance of determining the proper synchronization protocols as well as timing of insemination for each specific species. The time of AI is especially important in the case of sex-sorted sperm, since it has been demonstrated that insemination with sorted sperm closer to expected ovulation yields the highest probability of pregnancy in cattle and deer [53–55]. Therefore, it

Table 1
Lambing rates after laparoscopic insemination of synchronized East Friesian ewes (n = 285) with 60 $\times$ 10^6 total frozen-thawed conventional, 3 or 6 $\times$ 10^6 total frozen-thawed, and 1 or 2 $\times$ 10^6 total fresh X-chromosome bearing sex-sorted ram sperm (n = 2).

Type/dose of semen	Ewes Total	Lambing rate (%)	Born/ewes lambing
Conventional Frozen 60 $\times$ 10^6	60	**41.8**	1.6
Sex-sorted Fresh 1 $\times$ 10^6	57	**35.8**	1.4
Sex-sorted Fresh 2 $\times$ 10^6	56	**43.6**	1.4
Sex-sorted Frozen 3 $\times$ 10^6 (pellet)	62	**32.8**	1.4
Sex-sorted Frozen 6 $\times$ 10^6 (straw)	51	**56.1**	1.3

Table 2
Pregnancy rates after laparoscopic insemination of synchronized Merino ewes (n = 293) with 25, 10 and 2 × 10^6 total fresh conventional, and 10 and 2 × 10^6 total fresh X-chromosome bearing sex-sorted ram sperm (n = 1).

Type/dose of semen	Ewes Total	Pregnant (%)	Fetuses/ewes pregnant
Conventional Fresh 25 × 10^6	36	**78**	1.6
Conventional Fresh 10 × 10^6	62	**69**	1.7
Conventional Fresh 2 × 10^6	64	**69**	1.6
Sex-sorted Fresh 10 × 10^6	62	**65**	1.7
Sex-sorted Fresh 2 × 10^6	69	**51**	1.4

would be reasonable to assume that the same principle applies to sheep and goats as well.

In the past year, inseminations using fresh sheep semen (2 × 10^6 sperm per dose) have confirmed that, when used under the appropriate conditions, sex-sorted fresh sperm at a low dose can achieve equal or superior fertilizing ability compared with conventional semen from the same ejaculates (Table 3). In these fresh semen trials, ewes (n = 210) were synchronized using CIDRs for 12 days, followed by an intramuscular injection of 350 IU of PMSG at CIDR removal. Ewes were inseminated by laparoscopy at 53–54 h post CIDR removal. Pregnancy status was determined using transcutaneous ultrasound on Day 50–51 post AI.

SexedULTRA™ ram sex-sorted sperm is now a commercial product and it is expected that it will become an important breeding option at the elite stud level as well as the commercial farm level in the next couple of years. Nevertheless, more trials are currently in progress to develop optimal synchronization protocols and determine the minimum sex-sorted sperm per dose that would allow a corresponding decrease in the associated cost per dose.

4. Caprine sex-sorted sperm

Goats have been linked to humans for at least 10,000 years [56]. Due to their great adaptability to different environmental conditions and high productivity, they have always been considered useful farming animals. In the last 50 years, global goat populations have increased by about 240%, while other livestock species have maintained or decreased their populations. Currently, the global goat population is over 1 billion [57,58]. The potential for goats to produce a sustainable supply of milk and meat for human consumption is undeniable. Goats are specially valued for the nutritional quality and health benefits of their milk [59,60]. In developing countries, goat milk has contributed to improved nutrition in rural populations, where dairy goats are increasingly becoming a profitable business for farmers. At the same time, consumption of goat cheese is increasing in developed countries.

Although the dairy goat industry has not yet become well developed in many countries, with an increased understanding of the value of goat milk, the market will gradually expand, and biotechnologies such as sex-sorted semen that provide over a 90% sex skew will gain more importance, as they will facilitate faster and more profitable herd expansion.

It was not until 2013 that one of the few publications regarding sex-sorted goat semen reported successful sorting and birth of kids after LAI with about 32 × 10^6 sperm per insemination of either sex-sorted or conventional sperm. In this report, sex-sorted sperm cryopreserved in straws presented a post-thaw motility of around 48%, but suffered a high deterioration during a 2 and 4 h incubation. Fertility was lower for sex-sorted sperm (38% versus 50% kidding rate for sex-sorted and conventional sperm), but the success of the technique was demonstrated [20].

Table 3
Pregnancy rates after laparoscopic insemination of synchronized ewes (n = 210) with 10 × 10^6 total fresh conventional, and 2 × 10^6 total fresh X-chromosome bearing sex-sorted ram sperm (n = 1).

Type/dose of semen	Ewes Total	Pregnant (%)	Fetuses/ewes pregnant
Conventional Fresh 10 × 10^6	98	**83**	1.7
Sex-sorted Fresh 2 × 10^6	112	**86**	1.5

With further optimization of the sex-sorting extenders, along with the implementation of SexedULTRA™ procedures and the use of state-of-the-art Genesis sperm sorters, the commercialization of caprine sex-sorted sperm became a reality at the end of 2015. Since then, over 20,000 sex-sorted straws have been produced for LAI purposes with a mean (±SEM) post-thaw visual sperm motility of 59.1 ± 1.1%, a computer assisted sperm analysis (CASA – IVOS II system) total and progressive motility of 66.0 ± 1.7% and 58.2 ± 1.8% respectively, intact acrosomes of 73.5 ± 0.9% and a female sex purity of 92.9 ± 0.3%.

In small scale field trials, 75 does were divided in two groups and inseminated with sex-sorted fresh (2 × 10^6 sperm per insemination) or sex-sorted frozen sperm (4 × 10^6 sperm per insemination). Pregnancy rates were 57% for fresh and 49% for frozen sperm. These results were comparable to conventional semen used in the same farm during the same period. A second trial including 100 does split in three groups and inseminated using LAI with conventional fresh semen at 20 × 10^6 sperm, conventional frozen semen at 20 × 10^6 sperm and sex-sorted frozen sperm at 2.1 × 10^6 sperm per insemination demonstrated comparable pregnancy rates (40%, 39% and 36%) between conventional and sex-sorted sperm.

The next challenge was to deliver the appropriate fertile dose of sex-sorted sperm to be used for transcervical inseminations. Although laparoscopic inseminations provide more reliable conception rates, they are also a surgical procedure that requires high standards of skill, asepsis and analgesia. In small ruminants, it is important to consider the anatomical structure and narrow cervical way through to the corpus uteri to determine the AI method to be carried out to increase pregnancy rates while maximizing animal welfare. It is well known that sheep are the most difficult to inseminate transcervically, while goats are the easiest, and deer are between the others in difficulty. The breed within a species is also important, for example, in goats, the anatomy of the Domestic Alpine goat allows for a deeper insemination into the cervix than in other breeds. One must also consider the type and quality of semen to be used, such as fresh or frozen, and high or low dose, to determine if transcervical insemination is an option. Studies have also demonstrated that the results of laparoscopic and vaginal insemination are highly dependent on the technician who carries it out [61]. In the first transcervical AI trial, a total of 624 does across two farms were inseminated with 60 × 10^6 conventional frozen sperm or with 4 or 8 x 10^6 sex-sorted frozen sperm per insemination. The results varied widely between farms, with Farm 1 presenting lower pregnancy results when using sex-sorted semen, and Farm 2 demonstrating comparable results between conventional and sex-sorted semen at 4 million per dose (Table 4). Since the semen used in both farms belonged to the same freeze codes, it is hypothesized that the lower pregnancy results in Farm 1 could be attributed to the timing of AI or the experience of the inseminators, although more trials including different breeds of goats are required to confirm the results.

5. Cervine sex-sorted sperm

The captive deer breeding industry has experienced an important period of growth worldwide in recent years [5,62]. There has been an increased trend toward deer gaming farms where the main financial profits rely on antler trophies. Given that males have the

Table 4
Pregnancy rates after transcervical insemination of synchronized Domestic Alpine does (n = 624) across two farms with 60 × 10^6 total frozen-thawed conventional, and 4 or 8 × 10^6 total frozen-thawed X-chromosome bearing sex-sorted sperm. Farm 1 presented decreased pregnancy rates when using sex-sorted semen, while Farm 2 presented comparable results between conventional and sex-sorted sperm.

FARM 1 - Type/dose of semen	Does Total	Pregnant (%)	Sex Purity (%)
Conventional Frozen 60 × 10^6	104	**61**	59
Sex-sorted Frozen 4 × 10^6	102	**13**	100
Sex-sorted Frozen 8 × 10^6	103	**35**	96
FARM 2 - Type/dose of semen	**Does Total**	**Pregnant (%)**	**Female Purity (%)**
Conventional Frozen 60 × 10^6	130	**61**	58
Sex-sorted Frozen 4 × 10^6	46	**62**	87
Sex-sorted Frozen 8 × 10^6	139	**44**	92

highest economic value, sex-sorted sperm represents significant management cost-savings.

Commercial production of cervine sex-sorted sperm started in 2009 in the headquarters for STGenetics® in Navasota (Texas, USA). Since then, there has been a steady market for both fresh and frozen sex-sorted sperm in white-tailed deer in the United States, amounting to many thousands of straws produced every year.

In vitro sperm quality studies comparing post-thaw motility and DNA fragmentation kinetics of sex-sorted and conventional sperm of red and white-tailed deer have shown equal or better semen characteristics for the sex-sorted samples [6]. A more recent *in vitro* quality analysis of white-tailed deer sperm including percent visual motile sperm, intact acrosomes, CASA for total and progressive motile and sex purity is part of the commercial production routine and demonstrates equal or better semen characteristics for the sex-sorted compared to conventional samples (Fig. 2).

Information on fertility of sex-sorted deer sperm is still limited but most reports point towards the resilience of red and white-tailed deer sperm to withstand the sorting process and maintain good fertility. Fertility trials with red deer using 3 x 10^6 sex-sorted sperm on two separate ranches showed that pregnancy results were similar using conventional and sex-sorted sperm [7]. Other studies show a slightly lower fertility of Y sorted sperm when using Iberian red deer [63], although pregnancy rates were significantly higher when hinds were inseminated closer to ovulation induction so, as with sheep and goats, lower fertilities could be attributed to the need of devising synchronization protocols for each specific species when using sex-sorted sperm samples [55].

There are no published reports on the application of SexedULTRA™ for LAI in deer, but personal communications from the sorting laboratories confirm that, when cryopreserved sex-sorted cervine sperm at a dose of 8–9 x 10^6 is used according to STGenetics® breeding management and synchronization recommendations, pregnancy rates are ~93–95% relative to those of conventional (70% vs 74%), while fresh sex-sorted sperm processed at 4 × 10^6 per dose is achieving average conception rates 5–8% better than those of conventional. Male purity in the field has been reported to be 92–95% for deer sex-sorted sperm (Personal communication in 2019. Jared Templeton, Global Production Manager. STGenetics®).

As with other small ruminants, there has been an increased interest in using transcervical AI in deer. A small-scale field trial was carried out in the 2019–2020 season, with a total of 244 does inseminated with 20 × 106 conventional frozen sperm or with 6, 8 or 10 x 106 sex-sorted frozen sperm per insemination. Estrous synchronization of does was performed using CIDR devices. Upon CIDR removal on day 14, does were injected intramuscularly with 200 IU of pregnant mare's serum gonadotropin (PMSG). Insemination of does started 58 h after CIDR removal. Does that had been inseminated were moved to a separate pen to be housed individually one week prior to the expected parturition date. Fertility rates were assessed based upon delivery rates. The results from this trial indicated that sex-sorted frozen sperm at either 8 or 10 × 106 achieved comparable results to those of conventional after transcervical AI, although the overall results were lower than those achieved with LAI at lower doses (Table 5).

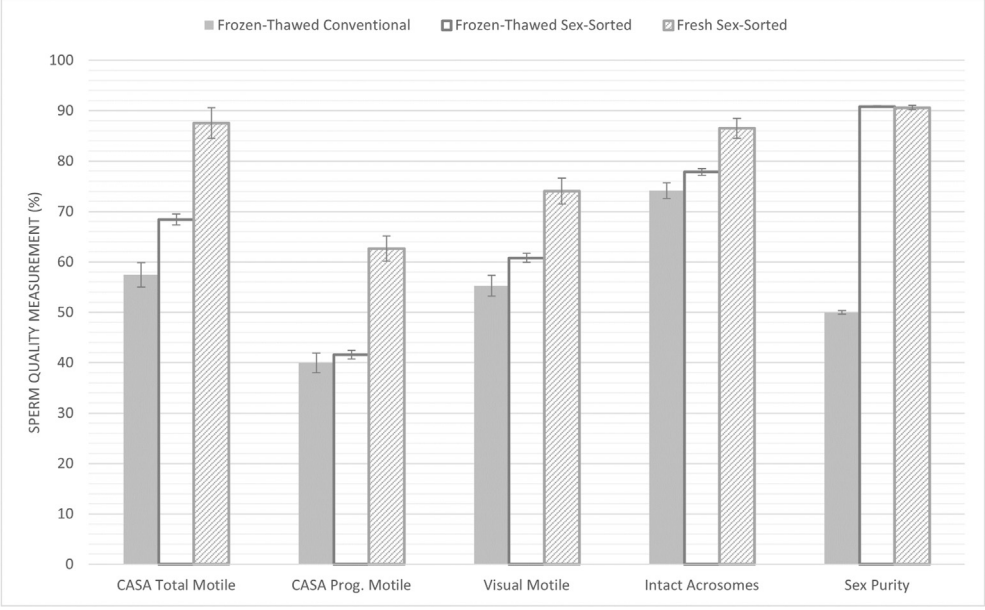

Fig. 2. Sperm quality least square means (±standard error) for all the white-tailed deer conventional (n = 32), sex-sorted frozen-thawed (n = 162) and sex-sorted fresh (n = 20) semen lots produced at STGenetics® in Navasota (Texas, USA) during 2017–2018 season. Percent CASA total and progressively motile, visual motile and intact acrosomes were measured 20 min after thawing the straws for frozen semen, and immediately after sorting and final dilution for fresh semen. Cryopreserved straws were thawed at 38.5 °C for 45 s. Cryopreserved sex-sorted cervine sperm was processed at a dose of 8–9 x 10^6 per 0.25 cc straw, fresh sex-sorted sperm was processed at 4 × 10^6 per 0.25 cc straw, and conventional semen was processed at 20 × 10^6 per 0.25 cc straw.

Table 5
Pregnancy rates after transcervical insemination of White Tail deer does (n = 244) across two farms with 20 × 10^6 total frozen-thawed conventional, and 6, 8 or 10 × 10^6 total frozen-thawed Y-chromosome bearing sex-sorted deer sperm (n = 4).

Type/dose of semen	Does Total	Calving rate (%)
Conventional Frozen 20 × 10^6	54	28
Sex-sorted Frozen 6 × 10^6	30	17
Sex-sorted Frozen 8 × 106	75	32
Sex-sorted Frozen 10 × 106	85	36

6. The future of small ruminant sperm sorting

In the past decade, sheep and goat production has increased by about one-third due to their economic value as efficient converters of low-quality forages into quality meat, milk, and wool [2,64]. The deer gaming farms have also experienced a period of growth worldwide [65].

For these growing industries, it is imperative to use all available pregnancies to modify the offspring sex-ratio to generate productive animals (females or males), allowing for faster genetic progress and increased production while reducing wastage. Sperm sex-sorting by flow cytometry is the only reliable technology to separate X- and Y- chromosome bearing sperm based on their difference in DNA content. The technology has now been validated for all three species on the basis of laboratory analysis and live births and incorporates modified flow cytometric sorting instrumentation and SexedULTRA™. Further research is still needed to determine the appropriate sperm dose for laparoscopic and transcervical AI, as well as the synchronization protocols and timing of AI that would provide results comparable to conventional semen when using sex-sorted sperm on each species.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

The authors are employed by STGenetics®/Sexing Technologies.

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