Sex-Sorted Sperm and Fertility: An Alternative View

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ABSTRACT

Although contemporary methods of physically separating X from Y chromosome-bearing spermatozoa are now very efficient, overall fertility rates following the use of sex-sorted sperm are not as impressive, in spite of many attempts to improve them. At the same time, there are suggestions from evolutionary biology, and from sex allocation theory in particular, that there may need to be a modification to the chance theory of sex determination in mammals. This is because it now appears that the mammalian female could have some influence on the sex of her offspring, and furthermore, that this influence could be preconceptual. If so, this could go some way towards accounting for the putative inefficiencies in fertilization following insemination with sex-sorted sperm.

INTRODUCTION

Beginning over 100 yr ago, with the discovery of the role of the chromosomes [1], and continuing to the present day, the allocation of sex in mammalian offspring has been considered to be a matter of chance, depending on whether an X or a Y chromosome-bearing spermatozoon (X or a Y sperm) reaches the ovum first.

At first assuming, and later showing, that X and Y spermatozoa are produced in equal numbers [2], early work on sex preselection focused on finding a measurable difference between them, especially one that would lead to a reliable way of sorting them into discrete groups. If this were possible, ensuring the conception of an offspring of a particular sex could be achieved by exposing the ovum specifically to Y sperm only (for a male) and not to X sperm, or vice versa. This thinking resulted in the emergence of the sperm separation industry.

ACHIEVEMENTS OF THE SPERM SEPARATION INDUSTRY

Early methods of sorting X and Y sperm were sometimes based on unwarranted assumptions about differences between them [3], resulting in less than adequate concentrations of the desired spermatozoa in sperm samples [4]. Contemporary separation processes are based on a sex difference in the amount of DNA in the X and Y chromosomes: a human X chromosome containing approximately 2.8% more total DNA than a Y chromosome [5]—the difference in other mammals being on the order of 3%–5%.

This difference made it possible to devise ways of more accurately separating sperm into X-enriched and Y-enriched samples than had previously been possible. Successful methods of separation, for use primarily in the dairy industry, involve flow cytometry/cell sorting after staining sperm with a fluorescent DNA-binding dye [6]. Now sometimes referred to as the Beltsville method [7], it has recently been even further refined by the addition of an orienting nozzle, which permits better alignment of the sperm during the sorting process, leading to further increases in efficiency and accuracy.

In general, contemporary sperm-sorting procedures result in skewed offspring sex ratios of between 85% and 95% accuracy [7]. Thus, in a number of different animal species, including sheep [8], dairy cattle [9], horses [10] and pigs [11, 12], once a pregnancy is established, the incidence of the unwanted sex is low.

THE FERTILITY PROBLEM

In spite of the remarkable technical expertise that has resulted in these achievements, the success rate, in terms of total numbers of live births, is not as impressive. Key researchers acknowledge that fertility of sorted spermatozoa is “somewhat lower” [13] than that of controls, but point out that results of studies showing this difference “are confounded by using fewer sexed spermatozoa per insemination dose than normal procedures would dictate” [13].

One of the major limitations of flow cytometric sperm sorting is the sorting rate, which means that animals fertilized with sex-sorted sperm tend to receive much lower doses of sperm than animals undergoing routine artificial insemination (AI). For example, in the horse, optimized flow-sorting allows approximately 10–20 × 10⁶ sperm to be sorted per hour; thus, it is impractical to obtain the 500 × 10⁶ sperm that would typically be used to inseminate mares with unsorted sperm [14]. However, when similar numbers of sorted and nonsorted spermatozoa were used in an insemination trial in cows, pregnancy rates with sex-sorted spermatozoa continued to be 20%–40% lower than control, unsorted spermatozoa [15].

There also appear to be differences between species in the fertility rates using sex-sorted sperm, with some species (e.g., sheep [8], horses [10], and pigs [12]) having low pregnancy...
rates following insemination with sex-sorted sperm compared to insemination with unsorted sperm. However, such interspecies differences may reflect suboptimal insemination procedures, as well as problems with the fertilizing potential of sex-sorted sperm.

Although it can be difficult to unearth figures that give the complete picture, reports from the dairy industry indicate a somewhat higher level of achievement. Figures are usually given in terms of percentage success rate of sorted sperm compared with that of unsexed samples in routine AI. The pregnancy rate following AI varies, but can be between 63% and 72% [15]. Thus, if the pregnancy rate for AI, using unsexed sperm, was 63%–72%, the predicted pregnancy rate for sex-sorted sperm would be 38%–58%. According to a recent review, fertility rates for sex-sorted sperm are now usually in the range of 70%–80% of unsexed sperm at normal doses in well-managed herds (although, the author adds, it has been “lower in poorly managed herds”) [16]. So even the predicted [16] average pregnancy rate for sex-sorted sperm in the dairy industry (70%–80% of 63%–72%) is about 51%, and can be considerably lower [9].

Some studies do give sufficient detail to make the situation clear. Following a field study in Switzerland, researchers reported fully on the results of inseminating heifers or cows with low-dose sex-sorted or nonsorted sperm [9]. For the combined group, results showed 85.3% female offspring for the sex-sorted sperm compared with 58.6% females for the nonsorted sperm, a result which, as in many earlier studies, confirmed the efficacy of the sperm-sorting procedure. However, closer inspection of the data reveals an interesting phenomenon. A total of 27 heifers received sex-sorted sperm and 27 heifers received nonsorted sperm, both at the low dose, and in similar conditions; but heifers that had been fertilized by nonsorted sperm had nearly twice as many calves as those inseminated by sorted sperm (15 and 8, respectively). In the second part of the study, cows were treated in the same way, but in this case, calving rates did not differ, with both groups having low fertility—22.1% pregnancy rate (23/104) in the group receiving sex-sorted sperm, and 23.4% pregnancy rate (16/63) in the group receiving nonsorted sperm [9].

At the present time, it is widely acknowledged that subfertility is a problem throughout the dairy industry [17], and this too should be kept in mind when considering fertility rates following sperm sorting.

ATTEMPTS TO IMPROVE FERTILITY RATES

Many of the industry’s most recent initiatives appear to be targeted at improving fertilization rates. All aspects of the procedures are being scrutinized and analyzed, including possible effects of the sorting process itself [7, 18], effects of transporting and storage of sperm [19], optimal concentration of sperm [20], possible effects of polyspermy [21], mode and timing of delivery of the spermatozoa [22, 23], and insemination site [10], but, so far, lower fertilization rates remain unexplained.

Lower pregnancy rates appear to be associated with the use of sex-sorted sperm at virtually every step in the process (i.e., lower fertilization rates tend to be followed, in addition, by lower cleavage rates and lower rates of development to blastocyst stage [20]). Whatever the cause or causes, the conclusion is similar across a number of studies; namely, that “sex-sorting sperm negatively impacts the embryo’s ability to develop normally” [23]. Thus, it could be argued that the procedures associated with the use of sex-sorted sperm are not yet fully refined, that results continue to improve [20], and that, along with generalized subfertility, this is a sufficient explanation for the low fertilization rates. Whatever the final outcome, there is no doubt that these are currently reasonable positions to hold.

On the other hand, if the basic model of sex determination is inaccurate, there may be a way of interpreting the data that shows fertility following the use of sex-sorted sperm to be much more successful than currently viewed. The disadvantage of this new interpretation would be that contemporary and emerging technologies might not contribute as much to efficiency gains as expected.

DIFFICULTIES INTERPRETING THE DATA

There are several reasons why overall fertility rates could be even lower than appears from an appraisal of the data. The first is the convention of reporting the number of pregnancies rather than the number of live births. Although times differ between species, there can be wide variation in attrition rates during pregnancy [10]. Some researchers specifically mention the possibility that pregnancy losses in cattle between 1 and 2 mo of gestation may be on the order of 1%–2% higher than those observed with normal insemination doses of unsexed spermatozoa [13, 22].

Secondly, while fertility rates following sperm separation continue to be given as a percentage of those following AI with unsexed spermatozoa, it can be difficult to estimate where the most important problems lie.

Thirdly, it could be argued that low fertility rates are underreported. Negative results are less likely to be published, and researchers less likely to report them, especially if it is then suggested that they are not managing their herds properly. The fact is, as key researchers have reported, fertility is “somewhat lower...even under ideal conditions such as highly fertile bulls, skilled AI technicians and well-managed heifers” [6].

Finally, low fertility rates may be less apparent in the literature because of the implicit assumptions in the theoretical model of sex determination. Methodologically speaking, if the accepted model of sex determination is that it is a matter of chance, then the number of females that fail to become pregnant is perhaps only of peripheral, academic interest (although of considerable commercial interest). On the other hand, with a model of sex determination that included a role for the mother, such information would be central.

THE STUDY OF OFFSPRING SEX RATIOS IN EVOLUTIONARY BIOLOGY

At the same time as research into sperm separation was being undertaken in andrology and reproductive physiology, evolutionary biologists were documenting atypical offspring sex ratios, both in the wild and in captivity, searching for a solution to the problem of sex allocation in mammals. In part, this work was an exploration of Fisher’s frequency-dependent sex selection theory [24], and its more specific successor, the Trivers and Willard hypothesis [25], which described how an adaptive advantage could accrue to a mother in good condition if she conceived and raised a male offspring.

In the last 30 yr, theorists and field workers alike have explored the mathematics of evolutionarily stable strategies [26, 27] and searched for evidence of adaptive control of the sex ratio in mammals [28–30]. In theory, especially since many nonmammalian species appear to have precise adaptive control of offspring sex ratio [31–33], mammals should have adaptive control too, not least for the advantage that such control would bring.

Furthermore, because the mammalian mother commits more energy to the growth and development of the offspring...
compared with the father, both pre- and postnatally, it would make sense for her to have some say in which sex offspring would be more likely to achieve the greater fitness benefit at any particular time.

Thus, over the years of searching, there has been an increasing emphasis on the possibility of a role for the mammalian mother. Trivers and Willard’s “good condition” was later found to be related to maternal dominance, a behavioural characteristic associated with good condition [34], and shown to be underpinned by testosterone in females [35, 36]. Both good condition and maternal dominance have been shown to be associated with the conception of statistically significantly more male offspring [37, 38].

More than 1000 research papers have now cited the Trivers and Willard hypothesis [37], and, although there are still problems [28, 39, 40], almost half the studies (48%) investigating the hypothesis found statistically significant alterations in secondary sex ratios in the predicted direction [37], and there have been various suggestions for refinements to the hypothesis that could help explain many, if not most, of the failures to confirm it [41, 42].

Out of concern for the apparent clash with the chance model of sex determination, some evolutionary biologists have argued that significantly different offspring sex ratios could be accounted for by maternally induced pre- and perinatal mortality by sex of offspring [43–46]. These include differential loss by sex during pregnancy, as well as sex-biased neglect or infanticide at birth. There is no doubt that these occur, but little evidence to support the suggestion that this could be a sufficient explanation for atypical sex ratios. On the contrary, there is now growing evidence to suggest that both avian and mammalian mothers could have a role in predetermining the sex of their offspring at, or before, conception. For example, researchers studying atypical sex ratios in the Seychelles warbler were led to surmise, counter-intuitively, that “pre-ovulation mechanisms may be the sole means of adjustment in this species” [31]. In a recent review of sex ratios in ungulates, data were found to be consistent with the predictions of the Trivers and Willard hypothesis, provided that the researchers had differentiated between pre- and postconception measures, with preconception measures being the accurate predictors [38]. Evolutionary biologists who have included hormonal measurements along with their behavioural data have also found evidence for preconceptional influences on offspring sex ratios from maternal hormones [47, 48].

In all this, maternal testosterone may yet prove to be central, because it has the potential to provide a link between the behaviours associated with dominance [35, 36, 49] and the physiological processes involved in conception [50, 51].

A POSSIBLE MATERNAL ROLE IN SEX PREDETERMINATION

Because atypical sex ratio data has been associated almost entirely with maternal characteristics (and not with paternal characteristics, which remain largely unknown), mid-20th century researchers suggested that the uterine environment might facilitate preferential access of either X or Y sperm [52]; however, it appears that there is no evidence to support this idea [4], although some form of maternal ratification of embryonic sex may yet be found to be relevant.

A more recent, alternative hypothesis is that an ovum may be selected and developed each oestrus or menstrual cycle, already adapted to receive an X or a Y sperm [53]. This could be the most parsimonious and least costly mechanism suggested so far. One hypothesized pathway by which this could occur is currently being investigated. It involves the follicular fluid, known to have concentrations of testosterone 10,000–30,000 times higher than serum [50]. Individual samples of follicular fluid may be either high or low in testosterone, and it is these differences, it is hypothesized, that could lead to differences in the development of the zona pellucida (ZP), such that it is rendered more or less easily penetrable by, or receptive to, either an X or a Y sperm. The molecular basis for such testosterone-induced selectivity might be very subtle. For example, it is known that carbohydrates on the ZP proteins form the oocyte receptor for sperm binding, and testosterone is known to influence the glycosylation of proteins [54]. Thus, it is possible that testosterone could also subtly alter the glycosylation on ZP3 and/or ZP2 such that one or both of these molecules preferentially accepted a Y sperm. This would mean that X and Y sperm might have demonstrable differences in their oocyte receptors. However, despite there being many candidate ligands for ZP3 on sperm, the primary ligand is currently unknown; thus, it is very difficult to confirm this hypothesis experimentally.

Overall, however, the idea that mammalian mothers could have a role to play in the predetermination of the sex of their offspring is consistent with theoretical work [26, 55–57], field work [58], and laboratory work [53, 59]. If it transpires that follicular testosterone is, in part, responsible for differential development of the ZP each oestrus or menstrual cycle, this could provide a pathway for the mother to adjust the sex of the offspring to suit current conditions. This is because testosterone levels (in both males and females) are sensitive to chronic environmental stressors: male testosterone falling, but female testosterone rising as part of a female mammal’s intrinsic response to stress [49]. It could be that normally distributed female testosterone, known to fluctuate over time in response to environmental stressors, might also provide a clue to solving the problem of adaptive control of the sex ratio in mammals.

IMPLICATIONS FOR SEX SELECTION BY MEANS OF SEX-SORTED SPERM

So far, research into the efficacy of sex-sorted sperm appears to be consistent with the possibility of a maternal role in sex predetermination. Many studies have been published showing that, in spite of the most careful and consistent application of the techniques, fertility appears to be compromised. From this viewpoint, studies in which success rates are reported only in terms of numbers and sexes of live births, but do not mention the numbers of mothers that failed to become pregnant at all, do not give the information required to make a proper assessment of the efficacy of the procedures.

A further question arises from the current convention of reporting identifiable pregnancies instead of live births, since another unknown factor is the point at which an embryo of a particular sex might need to be compatible with, or possibly, ratified by, the uterine environment.

At present, it is difficult to determine whether the low fertility rates are best attributable to factors associated with the sorting process (including damage to sperm), problems with insemination techniques, or low doses. Experiments using matched doses of sorted and nonsorted sperm attempt to address the issue of total numbers of sperm used, but even these experiments cannot control for the confounding effects of sperm damage (such as premature acrosome reaction [60]) that are almost certainly introduced by the sorting process. To perform a critical trial of sperm sorting, instead of comparing sorted and unsorted sperm, the fertilization rates of sorted and sorted/recombined sperm should be compared. This experi-
mental design would control for both low dosage and sperm damage. However, to our knowledge, this definitive experiment has not been carried out.

If, on the other hand, the ovum has a role in sex allocation, then no matter how technically successful the sperm sorting techniques, fertility rates will remain low. That is to say, if an ovum is produced, each cycle, already adapted to receive an X or a Y sperm, exposure to any number of the other kind of sperm will result in failure to fertilize or failure to develop. This would mean that sex-sorted sperm were useful only when a client wished to have, say, female offspring, or nothing.

With further refinements to sperm-sorting procedures, it is still possible that the fertilizing potential of sex-sorted sperm could be high. However, an alternative explanation for the low birth rates might be the one offered here, that the fault lies in assumptions about the model of fertilization, since the ovum may be playing a role in predetermining the sex of the offspring. Controversy and doubt surround this suggestion, but, if true, it is a researchable question. Are fertility rates following sperm separation compatible with the hypothesis that there is a preconceptual, maternal influence on the allocation of sex in mammals, or will they, ultimately, confirm the chance model of sex determination?

REFERENCES


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