SEXING MAMMALIAN SPERM — OVERVIEW

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THE NEED FOR SEX CONTROL

Being able to preselect the sex of offspring at the time of conception ranks among the most
sought-after reproductive technologies of all time. This ability runs counter to trends of nature
which has gone to extreme lengths to make X- and Y-chromosome-bearing sperm phenotypically
identical so that mammalian sex is determined randomly, with equal chances of male or female
offspring (18). However, from an individual’s (or couple’s) perspective, the sex of the next child
born often matters, as does the sex of offspring to owners of certain individual domestic animals.

In the year 2000, the situation with regard to sex selection is fundamentally different from
what it was 50 or 100 years ago. We illustrate this with 4 examples.

Perhaps the most important application of sexing is to minimize sex-linked genetic disease
in the human population. There are more than 500 known X-linked diseases, some of which are
extremely debilitating. In most cases, half of the sons born from female carriers of X-linked
genetic disorders will be affected, while daughters will not. Thus, sex preselection to increase
the chances of having a female child gives the couple reasonable assurance that they can avoid
expression of the disease in their offspring (7). A less compelling application is sex selection for
purposes of family gender balancing. Even so, parents with at least one child of a particular sex
could decide for the opposite sex so as to balance the sex of family siblings. This more
controversial application of sex preselection in human beings, nevertheless, has considerable
potential for decreasing population growth (19) in a non-coercive manner.

Over the past century, husbandry practices in animal agriculture have changed dramatically
in developed countries. Sexing becomes particularly valuable with the systems of maternal and
terminal-cross lines that have arisen recently. Inefficiency always has been a dilemma with sex-
limited traits such as milk production and producing the mothers of the next generation (where
males are a by-product), but it now also is an issue with sex-influenced traits such as more
efficient growth of male beef animals (9). We are seeing dramatic changes in swine production
with consolidation and vertical integration that affects how swine are produced and marketed.
The use of sex preselection has great potential for increasing efficiency. In the future, those not
taking advantage of efficiencies such as sex control may be buying animal products from those
who do. Just as we have seen major changes in markets associated with animal agriculture over
the past 25 years, we no doubt will see similar changes in the future. With a world population now over 6 billion and having doubled in the last 30 years, the demand for efficient production of animal products will only increase.

A third choice available in 2000, which was not even considered in 1900, is assisted reproduction in endangered species. We are now more aware of and more responsive to extinction rates, and there are technological fixes available for endangered species in a few cases. If only a few individuals of an endangered species are available, chance production of a few males instead of a few females often would greatly diminish chances of survival of the species. Sex preselection for particular matings also becomes crucial in developing breeding systems that minimize inbreeding with few breeding animals.

A final example is the currently vast number of people who directly or indirectly spend considerable sums on companion animals. Horses provide the best illustration in North America. In many cases, horse owners want a specific sex from a given mating simply out of personal preference, and they often are willing to pay for the preference. Of course, there often will be an economic incentive as well, for example in producing female polo ponies.

These 4 examples of early 21st century reality are not meant to be exhaustive, and they do not apply to all countries and all cultures. They do illustrate, however, that demand for selection of sex at conception is increasing.

THE SCIENCE OF REGULATING SEX OF OFFSPRING

As with many scientific and technological objectives, a spectrum of approaches to controlling sex has evolved. In many species, it is possible to determine sexes of fetuses with relatively non-invasive use of ultrasound. This has limitations; for example, it is inaccurate earlier than 2 months of gestation in cattle, and difficult physically after 3 months of gestation because of the position of the fetus. Furthermore, sex is identified, not selected, unless one uses the inefficient route of selective abortion. However, identification of sex provides exceedingly useful information for planning and marketing purposes, and there is considerable demand for this service in cattle, horses, and in the human population. Of course, selective abortion for human applications is abhorrent in most human cultures.

The sex of preimplantation embryos also can be diagnosed fairly accurately with a variety of procedures (18). As with fetal sexing, identification rather than selection of sex is being practiced. However, discarding unwanted preimplantation embryos is not very costly and serves many purposes including avoidance of gestating offspring that express sex-linked genetic diseases. Furthermore, clever methods of simplifying procedures for sexing embryos are becoming available (e.g. 4). Of course, expenses of embryo transfer go along with this approach.

A very different set of approaches to regulating sex ratios is represented by timing mating relative to ovulation. While this has been advocated for years, it simply has not been repeatable (16). However, these "folkloric" methods never really had much chance of success because tools
for timing ovulation, or even knowing when it occurred in a given female, have not been generally available. Recent studies with more precise technological control of the reproductive cycle demonstrate that a small degree of distortion of sex ratio may, in fact, be possible in some species by timing insemination (16). A troubling aspect of these studies is lack of evidence of a plausible mechanism.

A related phenomenon is the seeming ability of female mammals of some species to regulate the sex ratio of their offspring to some degree according to environmental conditions (16). Recent evidence of different rates of development between male and female embryos under certain in vitro conditions does provide a plausible mechanism for such sex selection (16).

Finally, we come to the main subject of the symposium at hand, separating X- and Y-chromosome-bearing sperm. More than a dozen approaches based on perceived or assumed differences in physical characteristics or immunological factors have been attempted over the years (2, 12). One of these procedures, swimming of sperm through sperm albumin (6), has been used for many years in human fertility clinics in an effort to distort the sex ratio of children. However, the method has not been effective for use in animal studies nor has it been fully validated in humans with a properly controlled double-blind clinical trial. Its use remains a subject of controversy.

A new approach based on immunology is described in this symposium (3). Immunological approaches are highly desirable since they offer hope for inexpensive batch processing for sexing sperm. The conventional approach to immunological sexing, utilization of a surface protein, has shown little promise of being successful (8) to date. Distinguishing between X and Y sperm on the basis of a difference in sperm volume also is offered as a potential means of separation (20). The efficacy of these new approaches remains unclear since they have not been tested in practice.

The basis for the only effective means of separating viable X and Y sperm at the present time is the well established difference in DNA content between the 2 sex chromosomes. Try as one might, no other sex-specific marker has surfaced that approaches the consistency of the measurable difference in the DNA content of X and Y sperm. Flow-cytometric separation of viable X and Y sperm (10) is now so well established that the emphasis is not on if or how well it works, but how can we harness new technological advances in high-speed cell sorting to make the process produce more viable X and Y sperm per unit time at the highest purity (11). The greater the sexual sperm production per unit time, the wider the application for animal and human use at the least cost (1). Methods for processing sperm in parallel (8) rather than in series, however, would have a huge advantage. Further, no amount of technological advance can eliminate the fundamental disadvantages of interrogating each individual sperm by flow sorting and the necessity to add a dye to the sperm suspension in order to detect DNA differences. However, even with the disadvantages, its application in animal production (11, 14, 17, 19) and for humans (7) is essentially guaranteed.

Advancing the technology of sex preselection based on DNA content was not only dependent on a method of X/Y separation, but also an effective laboratory method for validating the putative populations of separated X and Y sperm. Prior to 1982, there was no
practical laboratory method of determining if any procedure for separating X and Y sperm was, in fact, successful other than inseminating females and determining the sex of fetuses or offspring. In other words, we could not even identify whether a sperm carried the X or Y chromosome. Now in 2000 we have several very credible procedures for verifying sex ratios at the sperm level. Sort reanalysis for DNA content by flow cytometry, fluorescence in situ hybridization (FISH) and use of the polymerase chain reaction on individual sperm are all effective for assaying samples for the proportions of X and Y sperm (22). Sexing embryos following IVF can be used post fertilization to accomplish the same purpose (18).

In this symposium, there is considerable emphasis on procedures leading to conception with reduced sperm numbers (7, 13, 15, 17, 19). Although the limited numbers of sperm available with flow-cytometric sperm sorting can be circumvented by using IVF or injection of sperm into oocytes (14), these are likely to be minor avenues for sex regulation relative to AI, conventionally (7, 19) or via laparoscopy (5) or surgery (13). It is likely that even "parallel" methods of separating X and Y sperm will not easily be adaptable to produce the numbers of sperm currently used for routine AI (19). Thus, reducing sperm numbers per pregnancy is likely to be necessary for application of most, if not all, potential sperm sexing procedures (1); this also obviously is of importance outside of the context of sexing.

SEXING SPERM VERSUS OTHER REPRODUCTIVE TECHNOLOGIES

In some respects, arranging for sex determination at conception is trivial compared to more sophisticated technologies such as transgenesis or cloning by nuclear transfer. But it is precisely the elegant simplicity that is so attractive. Sex differences not only are natural, but differences in performance of the 2 sexes (5 sexes if one includes castrates, spays, and freemartins) are relatively clearly quantified already. In contrast, abnormalities are common with many transgenic lines, and with many individuals produced by cloning and IVF. Clearly there is the caveat that any sexing procedure itself could cause abnormalities in offspring, and this concern must be addressed. While there is no evidence of an increased incidence of abnormalities in offspring conceived with flow-sorted sperm (10, 19), neither is there rigorous epidemiological evidence that a low level of induced abnormalities does not exist.

A concern often raised is that cloning will replace sexing sperm as surely as sexing sperm will replace sexing embryos. There is no question that technologies will supplant one another to some extent at some future time. However, market share of a given technology will be heavily dependent on costs, convenience, efficacy, acceptance by the public, absence of trade barriers, etc. With cloning by nuclear transfer, for instance, although one obviously gets automatic sex selection, efficacy is extremely low (but improving rapidly); pregnancies and resulting offspring often are abnormal (but it may be possible to circumvent this); and the real value of cloning in animal breeding often is vastly overrated (21). Except for traits of very high heritability (which excludes most agricultural production traits), applicability of cloning suffers both from the difficulty of identifying animals that truly are exceptionally superior genetically, and from the low repeatability of expression of production characteristics both within and between genetically identical animals (21). This is not to imply that cloning will not be an exceptionally valuable
tool, but rather that for routine production agriculture, the advantages may be less than they first appear, and costs for wide application, including embryo transfer, cannot be much higher than those of routine AI. The same is true for transgenesis, sexing sperm, or any technology, i.e. wide application for production agriculture must be efficacious, convenient, and inexpensive (9). A major limitation of sexing sperm is that additional technologies, such as artificial insemination or in vitro fertilization plus embryo transfer, must be used to produce the end product.

Another correction in thinking is that sexing embryos may become obsolete. There will be niche applications of genotyping embryos (for example, for marker-assisted selection), and sex can be determined relatively inexpensively as part of that process. If the embryos of one sex are very valuable, even 90% accuracy with sexed semen may be inadequate, and it will be worth sexing embryos to eliminate the 10% of the undesired sex.

CONCLUSIONS

Sexing sperm is a tool that has matured sufficiently to be valuable for experimental purposes and for certain niche applications relating to human health, human wants, and production-animal agriculture (7, 9). For wide agricultural application, costs must be low, and efficacy and convenience, high. Even when these conditions are met, sexing semen will remain a tool, not a panacea, and will be used along with other tools; in many cases, there will be synergy among technologies; examples include making AI justifiable on a much larger scale than current usage, and making cloning, IVF, and transgenic systems more efficacious. Furthermore, making sexed sperm practicable has been an intellectual challenge with important spinoffs including more efficacious cell sorters, new insights into immunology, and more efficient use of semen in AI. We look forward to further advances in this field.

REFERENCES