Transactions Natl. Acad. Sci. & Tech. Philippines 25 (2): 345-356 (2003)
ISSN 0115-8848

SEX PRESELECTION IN ANIMALS: CURRENT METHODS AND APPLICATIONS

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Abstract

Advancement in sexing technologies when used with other animal reproductive technologies presents opportunities to boost food production from animal sources and thus increases the availability of animal protein in the Filipino diet. The ability to predetermine the sex of offspring before and after fertilization of the ovum would allow farmers to raise animals of the desired sex based on their breeding needs and market demands. This paper reviews current sperm sexing technologies such as flow cytometry or cell sorting, H-Y antigen detection, and detection of sex-specific proteins on the sperm surface as well as embryo sexing technologies such as chromosome analysis, polymerase chain reaction and other methods. Moreover, it discusses how sexing technologies can further enhance other reproductive technologies namely artificial insemination, embryo transfer, in vitro fertilization, embryo splitting and cryopreservation, and the potential applications of these technologies in animal production.

Key words: DNA, embryo, flow-cytometry, sex chromosomes, sperm

Introduction

Recent advances in sexing technology present numerous opportunities for the improvement of the animal industry in both developed and developing countries.

The ability to preselect the gender of the offspring before and after fertili-

zation with appropriate sperm and embryo sexing technologies will have a great impact on animal production management systems as well as genetic improvement programs. Planned matings for a specific sex would then become feasible which would allow farmers to raise animals of the desired sex, and thus contribute to the improvement of production efficiency and product quality (Johnson, 2000). It will also contribute in many ways to increase profitability of livestock operations (Hohenboken, 1999). Because of the many potential benefits that sex preselection would bring to the animal industry and to mankind, mammalian physiologists have spent many years developing technologies that could be put into commercial use. In a few years (year 2005), it is predicted that sexed sperm of various animal species would be marketed in a commercial scale (Amann, 1999).

This paper reviews primarily the current methods of sex preselection using sperm and embryo of animals and explores the impact of these current sexing technologies on existing reproductive technologies such as artificial insemination, embryo transfer, in vitro fertilization, embryo splitting and cryopreservation. Moreover, the potential applications of sexing technology to animal production agriculture are also discussed.

The XY System as a Basis for Sex Determination in Mammals

Mammals have two separate sets of chromosomes whose genes determine sex. The system of chromosomal sex determination is through the XY system in which females possess XX chromosomes which are homologous and males possess XY chromosomes which are not homologous.

Current theory states that it is the Y chromosome that determines sex through the induction of testis development by a gene or genes termed SRY which is located on the Y chromosome. The Y-encoded testis determining gene has been named testis determining Y chromosome (Tdy) in mice and testis determining factor (TDF) in humans. The TDF stimulates embryonic gonads to produce testosterone which is responsible for differentiation of embryonic gonadal tissues into "male" structures. When the SRY gene is absent, female tissues develop (Hunter, 1995; Jafar and Flint, 1996).

Sex Preselection Approaches

The primary approach to sex preselection is through the sexing of spermatozoa before fertilization of the ovum. An alternative approach to sex preselection is sexing the embryo following fertilization. The current methods of sexing sperm and embryo are as follows:

Sperm sexing methods

Using this approach, sex is determined by separation of the Y- and X-bearing sperm.

1. Flow cytometry/cell sorting

This technique requires the staining of the sperm with Hoechst 33342, a binding dye which measures the DNA (deoxyribonucleic acid) content of individual sperm. The stained spermatozoa are then processed through a flow cytometer/cell sorter which separates the X- and Y-bearing spermatozoa based on the fluorescence exhibited by the sperm.

In most mammals, the X chromosome carries more DNA than the Y chromosome (Moruzzi, 1979), thus cell sorting is possible. The separation of the X- and Y-bearing sperm by flow cytometry was first reported by Johnson et al (1987). The use of sexed sperm resulted into the live births of rabbits of the predicted sex (Johnson et al, 1989). Live births from flow-cytometrically sorted sperm were also reported in cattle (Cran et al, 1993, 1995; Seidel et al, 1999) and swine (Rath et al, 1997). In humans, Fugger (1999) reported his successful clinical experience with flow cytometric separation of X- and Y-chromosome bearing sperm resulting in pregnancies and births of babies of the predicted sex.

The advent of newer generation sorters could put sexed sperm into commercial use in the next few years. The use of high-speed sorted spermatozoa already resulted into live births of the predicted sex in pigs (Abeydeera et al, 1998; Rath et al, 1999). This modified sorting system (high-speed cell sorter) can produce 6 million X-sperm and 6 million Y-sperm per hour (Johnson, 2000) in contrast to the old class of sperm system sorters called standard speed system which could only sort 350,000 sperm/hour (Johnson et al, 1989). Thus, the greater the sexed sperm production per unit time, the more it will become more practical for routine use because of lower cost (Amann, 1999).

Currently, credible techniques are available for verifying the sex ratio of sorted sperm. These laboratory methods include sort reanalysis of DNA by flow cytometry (Welch and Johnson, 1999), use of polymerase chain reaction (Welch et al. 1995) and fluorescence in situ hybridization (Karawasaki, et al. 1998)

The cell sorting technique is the most accurate and most promising method of separating the X- from the Y-bearing sperm. The main drawback is that it requires highly specialized, non-portable equipment which is quite expensive for routine use.

2. Detection of H-Y antigen on the surface of male somatic cells using antibodies

H-Y antigen is a Y-linked histocompatibility antigen expressed on the plasma membranes of male somatic cells. The theory on H-Y antigen was first reported

by Eichwald and Silmser (1955). The theory was based on the result of their skin graft experiment involving an inbred line of mice wherein skin grafts transferred from one female to another female were accepted, whereas skin grafts from a male to a female were rejected possibly due to induced immune reaction. This technique is not accurate because both X- and Y-bearing spermatozoa are H-Y positive.

3. Detection of sex-specific proteins

This technique is based on the theory that X- and Y-chromosome-bearing spermatids express X- and Y-chromosomal genes that might result in differences in protein composition of X and Y sperm. This means that a protein unique for X and Y sperm might exist. Hendriksen (1999), however, was unable to show the existence of such membrane proteins.

On the other hand, Blecher et al (1999) reported that sex-specific proteins (SSPs) could be detected on the plasma membrane of sperm cells. The SSPs were found to be more highly evolutionarily conserved than non-SSPs. They further showed that purified SSPs could be obtained and that sex specific antibodies against the SSPs seem to bind sex-chromosome specific proteins which would make X and Y separation by immunologic means possible. This study, however, is still in its initial stages of development and may take time to perfect the technique.

Embryo Sexing Methods

This approach primarily involves the examination of embryonic cells for sex chromosomes in order to determine the sex of the embryo.

1. Chromosomal analysis (karyotyping)

This cytogenetic technique involves preparing a chromosome specimen and examining the composition of the sex chromosomes to determine the sex of the embryo.

In the bovine species, their chromosomes consist of acrocentric and metacentric chromosomes allowing for easier morphologic identification of the sex of the embryo provided chromosome specimens have been well prepared. Thus, this technique is a good and accurate method of sexing bovine embryos.

Previous reports show that the chromosomal analysis method has been used successfully for sexing trophoblast cells of 11- to 15-day-old intact bovine embryos free of the zona pellucida (Hare et al, 1976), 6- to 7-day old intact bovine embryos (Singh and Hare, 1980) and 6- to 8-day old demi-embryos (bisected embryos) (Rall and Leibo, 1987). One disadvantage of cell karyotyping, however, is that some embryonic cells are destroyed during biopsy which re-

duce the viability of the embryo.

2. Polymerase Chain Reaction (PCR) sexing method

PCR is a technique for amplifying specific regions of DNA by multiple cycles of DNA polymerization, each followed by a brief heat treatment to separate complementary strands using a PCR machine (Alberts et al, 1994).

Recent reports show that PCR is an accurate method of sexing embryos. Chrenek et al (2001) reported that successful sexing of 75 preimplantation bovine embryos (16- to 32-cell stage) using multiplex PCR could be done. Only a single blastomere from each embryo was used for the experiment which showed a 91% sexing efficiency based on the amplification of Y-specific locus using K-casein internal standard. A rapid and reliable PCR method for the sexing of 8- to 16-cell stage bovine embryos was also reported by Park et al (2001). Using groups of 8, 4, 2, and 1 blastomeres for sexing, the efficiency rate obtained was 100.0, 96.3, 94.3 and 92.1%, respectively.

However, most PCR sex determination protocols include electrophoresis which is time consuming, but a simplified nonelectrophoretic PCR-sexing of bovine embryos has recently been reported (Husler et al, 2002). Based on this method, the sex determination rate obtained were 98.7% and 94.4% for male and female embryos, respectively.

The PCR is a fast and reliable technique for sexing embryos. However, the PCR machine is expensive.

3. Other methods

These methods have not been widely accepted for use in sexing livestock embryos because of low accuracy rates.

a. Detection of male embryos using Y-specific DNA probes

Leonard et al (1987) reported the sexing of bovine embryos using fragments of DNA that are found only on Y chromosomes. Thus, a DNA fragment that is male specific can be used as a probe for detection of male DNA fragments.

b. Detection of H-Y antigen using antibodies

Previous reports show that H-Y antigens were present from 4- cell to blastocyst stage mouse embryos (Krco and Goldberg, 1976). In the case of the bovine species, H-Y antigen appeared from the 16-cell to expanded blastocyst stage embryo.

Up to the present time, the sexing of bovine embryos using H-Y antibodies has not yet been perfected.

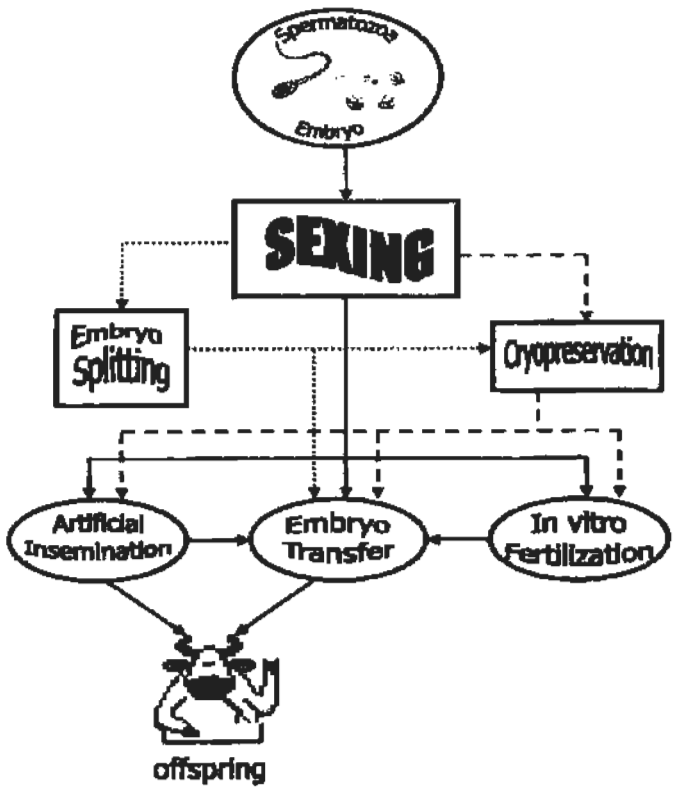

Figure 1. Impact of sexing preselection technology on other technologies of animal reproduction.

c. Detection of Barr body.

Barr bodies are sex chromatin bodies which have been detected in domestic rabbit blastocyst (6-day old embryo) and have been reported only in female embryos (Gardner and Edwards, 1968). However, this technique is difficult to use for livestock egg cells because of the presence of chromatin bodies in both male and female embryos.

The Impact of Sexing Technology on Other Reproductive Technologies

Sexing is a reproductive tool, which gives farmers a lot of opportunities and options for more efficient animal production. To maximize the benefits that could be obtained from such technology, it must be incorporated into other reproductive technologies such as artificial insemination (AI), embryo transfer

(ET) and in vitro fertilization (IVF) programs. The sexing technology could trigger the massive application of the above-mentioned technologies since farmers are now able to obtain valuable offspring of the desired sex (Figure 1).

For better clarity of discussion, these existing animal reproductive technologies are herein defined:

AI is a first-generation reproductive biotechnological technique in which spermatozoa are deposited into the female reproductive tract by mechanical means using an AI gun rather than by natural mating with a male. Fresh or frozen spermatozoa are usually used for AI.

ET is a new biotechnological technique by which embryos are recovered from valuable superovulated females (donors), and then transferred to surrogate mothers (recipients) that carry them till the end of gestation. Fresh or frozen embryos could be transferred to suitable recipients.

IVF is also a new biotechnological technique involving the fertilization of mature oocytes with valuable spermatozoa in an artificial environment (in vitro) which is usually performed in an IVF laboratory. This technique is a good means of producing embryos for ET work. In vitro produced embryos could be cryopreserved for future use.

The ultimate aim of AI, ET and IVF technologies is the genetic improvement of livestock. AI is practiced widely throughout the world but remains untapped in the country. The rate of AI usage (Valdez, 1993) has remained low especially in backyard farms. However, the advent of sexing technology could change the scenario when farmers are able to produce offspring of the desired sex. Generally, under local conditions, a farmer needs or prefers more female animals over that of males especially if he is engaged in a breeder type operation. While AI takes advantage of superior genetic material from the male side, ET allows the rapid multiplication of superior offspring from the female side, thus ET should also be tapped for genetic improvement. Although ET is being applied only in some university research institutions and other research stations, it is a technology waiting to be tapped to hasten livestock improvement in many countries in Southeast Asia (Kanagawa et al, 1990). IVF is also a technology being applied locally for research purposes, but could soon be of more practical value especially if sexed sperm is used to fertilize oocytes in vitro for the production of sexed embryos. In the long term, IVF could become an economical technique for animal improvement since it has been reported (Lu et al, 1999) that lesser number of sexed sperm is needed to fertilize an ovum in vitro compared to the volume of sexed sperm used for AI.

Other technologies of utmost importance are cryopreservation and embryo splitting of sexed sperm or sexed embryos. Cryopreservation is a technique of preserving sperm or embryo at low temperature by cooling in liquid nitrogen (-196°C). When a sexed sperm or sexed embryo is cryopreserved and stored in a liquid nitrogen tank, then a reserve of superior genetic materials will always be available for use by farmers anytime of the year for animal reproduction pur-

poses. Hence, the need to develop reliable and simplified freezing techniques for sexed semen or embryos for routine on-farm use (Valdez, 1996). Moreover, when a sexed embryo is bisected or split into two and cryopreserved for future use, then the practical value of sexing is amplified to the benefit of the animal producer. Embryo splitting is a technique of cutting or dividing embryos into halves using a micromanipulator. This is a good method of producing identical twins from sexed embryos.

It is thus apparent that sexing is a technology that cannot stand alone. It must become an integral part of AI, ET and IVF programs. But it is a technology that could be very promising and attractive to farmers because it allows them to preselect the sex of animals they are going to raise based on their needs. Technologies such as sexing will only succeed when farmers can use them profitably.

Potential Applications of Sex Preselection in Animal Production

The potential applications of sex preselection herein summarized are based on previous reports (Seidel, 2003; Seidel and Johnson, 1999)

Production of food animals (e.g. cattle, buffaloes, sheep, goats and swine) based on breeding needs and market demands

Livestock farmers are often disappointed when not able to produce offspring of the desired sex that are needed in their production operation. Generally, under local condition, there is a lack of female breeding stock which is needed to produce offspring for either dairy or beef production, thus females are preferred over males. However, when the scarcity for a particular sex has been achieved, then farmers would like an animal that could deliver the major goods they need. For instance, in dairying, females are usually desired for farmers to produce milk. When beef is the major product, then males because of their fast growing rates are preferred. The local cattle and buffalo industries would tremendously benefit from the application of sexing technology to boost the production of meat and milk.

Breeding of companion animals (e.g. dog, horse, etc.) based on the sex preference of the owner

This is an human driven application of the sexing technology wherein pet lovers and owners are able to preselect the sex of the next offspring of their animals based on their emotional needs or wants.

Assisted reproduction of endangered animal species

Depending on the need for a particular sex to raise, either more males than females or vice versa, or an equal number of the sexes (gender balancing) are bred and raised to conserve a particular endangered species (e.g. Philippine

Tamaraw, Philippine Tarsier, etc.). This is made possible with sexing technology wherein the animal breeder is able to devise a long-term breeding plan.

Research

Sexing technology is a powerful research tool for the study of sex-influenced traits in animals used for food production. It is also a potent tool for the study of gender-linked animal diseases as well as fertility and infertility problems.

Conclusions and Recommendations

There is no doubt that current advances in sexing technology to predetermine sex of livestock will revolutionize the development of animal industries worldwide. Hence, it is very important to keep abreast of such advances in both sperm and embryo sexing technologies in order that in due time, they could be adopted and incorporated as an integral part of existing technologies of reproduction such as AI, ET and IVF. The adoption of relevant sexing technologies in the foreseeable future should be looked into to boost animal production and to make available more animal protein for the Filipino populace. However, based on this review paper, unless fast, reliable, accurate and inexpensive sexing techniques become available in the near future, their practical application value may be limited. Therefore, local research efforts on sexing must be encouraged and directed towards simplification of current techniques for practical application.

Acknowledgment

The author is grateful to Dr. Salcedo L. Eduardo, Dean, College of Veterinary Medicine, University of the Philippines Los Baños (UPLB), for his encouragement and support in the writing of this manuscript. Many thanks are also extended to Dr. Teresa Asuncion B. Morillo, former Assistant Professor, Department of Veterinary Clinical Sciences, College of Veterinary Medicine, UPLB for her valuable help in providing research materials on sex preselection.

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