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Preselection of Offspring Sex at the Time of Conception in Mammals

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ABSTRACT

Background: There are currently numerous methods for sexing of sperms and embryos. The sex of zygotes is determined with the penetration of the mature oocytes by the fertilizing spermatozoa. The oocytes that are fertilized by spermatozoa bearing a Y-chromosome generally become males while the ones that are fertilized by spermatozoa bearing X- chromosome become females. Therefore, preselection the sex of offspring at the time of conception can be determined through the fertilizing spermatozoon or the transferred embryos. It considers the most sought-after assisted reproductive technologies (ARTs). **Objective:** The present review article addresses and discusses methods of preselection of offspring sex at the time of conception and their limitation and effects on mammals. **Results:** There are currently several methods of preselection of offspring sex at the time of conception in mammals, which differ in accuracy and complexity. The methods of preselection of offspring sex at the time of conception can be a great boon in both animals and humans as well. **Conclusion:** For the wide use of embryo sexing in mammals embryo transfer industry, a simple, rapid and precise sexing method needs to be developed.

INTRODUCTION

Assisted reproductive techniques have been developed in the last decade (Mohammed *et al.,* 2005; 2008; 2010; 2012; Mohammed and Attaai, 2011; Mohammed, 2014 a,b; 2016 submitted), which effect on human's life and prosperity. The techniques of preselection of embryo sexing at the time of conception can be a great boon in both animals and humans as well. It considers the most sought-after assisted reproductive technologies (ARTs). The ability to preselect the gender of the offspring before and after fertilization with appropriate sperm and embryo sexing techniques will have a great impact on animal production management systems, genetic improvement programs. The dairy industry generally prefers females for milk production whereas meat industry prefers males for meat production. In addition, pig industry generally prefers females due to lower cost of production and higher quality. The most important application of preselection of embryo sexing is to reduce sex-linked genetic disease in the populations of human. The patterns of inheritance are dominant or recessive, which are X-linked or Y-linked. More than 500 X-linked diseases are known. Half of offspring delivered from female carriers of X-linked diseases will be affected. Thus, preselection of offspring sexing gives the couples reasonable assurance that their offspring can avoid expression of diseases (Martinhago *et al.,* 2010).

Preselection of offspring sex at the time of conception is considered a desire in ancient cultures of different countries. The earlier approaches of preselection of embryo sexing was superstition based and the modern

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techniques make it possible. It was believed that the timing and position of sexual intercourse, mono-orchydectomy and diet affected the baby's sex (Schenker, 2002; Yan et al., 2006). Cryopreservation of embryos was carried out successfully and it followed by sexing of embryos through flow cytometry.

There are currently numerous methods for sexing of sperms and embryos. First successful embryo sexing was done by Gardner and Edwards (1968) by cytological methods (Barr body observation) in rabbits. Thereafter, progress in the recent years has been fundamentally achieved concerning to preselection of embryo sexing. The methods used in the world for embryo sexing are based on separation of X- and Y-bearing spermatozoa before fertilization (Aleahmad et al., 2009; Alkmin et al., 2014) or embryo sexing based on molecular of nuclear or mitochondrial DNA (Zoheir and Allam, 2011; Malik et al., 2013). Those methods are very expensive and time consuming. Embryo sexing using molecular basis necessitates aspiration of blastomere from the embryo, which adversely affect the developmental competence. Moreover, the success of *in vitro* fertilization using sexed sperm has been reported variation of a number of characteristics. Negative observations have included decreased cleavage rate (Zhang et al., 2003), lower blastocyst formation (Lu and Seidel; 2004; Bermejo-Alvarez et al., 2010), sire variation (Zhang et al., 2003; Lu and Seidel, 2004; Xu et al., 2006), and disturbed timing of development (Lu and Seidel; 2004; Blondin et al., 2009). Therefore, embryo sexing still necessitates a cheap noninvasive method to separate male and female embryos. The present review article addresses and discusses preselection of embryo sexing at the time of conception.

1. Embryo sexing through separation of sperm bearing X and Y chromosome:

The earlier approaches of embryo sexing were based on separation or sorting of sperm bearing X or Y chromosome. Many researchers have assessed enrichment medium methods to alter the sex ratio of mammals' spermatozoa. Many techniques have been developed to separate spermatozoa such as albumin density gradients (Classens *et al.*, 1995), modified swim-up procedure to enrich sperms bearing Y-chromosome (Check and Katsoff, 1993), sephadex column technique (Steeno *et al.*, 1975) and Percoll gradients (Iizuka *et al.*, 1987) to enrich sperms bearing X- chromosome and free-flow electrophoresis (Blottner et al., 1994; Ainsworth *et al.*, 2007). However, the proof of the enrichment of sperms bearing X or Y chromosome is controversial. Several studies (Welch and Johnson, 1999; Seidel and Gardner, 2002) suggested that flow cytometry to separate the X- and Y chromosome sperms showed a promising commercial potential to sort sperms. Unfortunately, the techniques have yielded inconsistent results and required appropriate skills and expertise as well as it is not easily accessible. Sperm separation occur according to specific traits in which spermatozoa are separated into Y- (male) and X- (female) chromosome based on their differences in DNA content. X-chromosome has more DNA than Y- chromosome; human 2.9; cattle 3.8; chinchilla 7.5 turkey 0. The sex-sorted spermatozoa can be used in conjunction with techniques of assisted reproduction as artificial insemination (AI) or *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) to produce offspring of the desired sex. The following figure indicates the methods used in sperm separation.

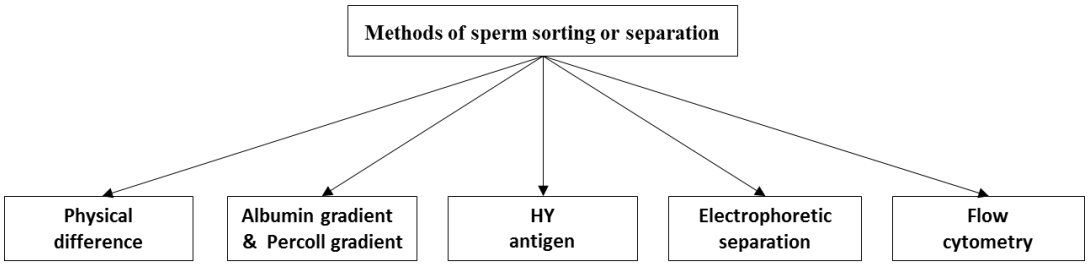


Fig. 1: Methods of sperm sorting or separation

1.1. Physical difference

Preselection of sex before conception requires separation of X- and Y- bearing spermatozoa. There are hypotheses based on supposed differences of physical characteristics of sperm as size and shape, density, and surface charge. Differences of sperm based on size and shape has not verified yet. The conclusions from experiments to verify separation of sperm bearing X- and Y- chromosome based on density were controversial. Electrophoresis for separating X- and Y-bearing sperm would be of little practical utility since sperm lost motility. Sorting spermatozoa through flow cytometry were based on DNA content and staining with Hoechst 33342 fluorescing DNA binding dye. The accuracy of sperm separation is approximately 90%. Sperm must be in the same orientation for detector. Thirty percent of spermatozoa only can be sorted for commercial purposes in cattle.

1.2. Albumin & Percoll gradients:

It has been claimed that sperms can be separated according to motility, density, size, chromosomal content and surface charges by using different washing methods. Albumin gradient has been used to separate sperms.

Trials that carried out using animal sperms gave contradictory results to confirm a final fraction rich in Y sperm. Paul Dmowski *et al.* (1979) separated husbands' semen using serum albumin gradients. The separated sexed semen was used thereafter for artificial homologous insemination (AIH). They reported upon using the separated sexed semen obtaining 6 male and 2 female infants out of eight pregnancies. The ratio of male to female conceptions resembles the ratio of Y to X sperm in the final specimen used for AIH in this study. Hadi and Al-Timimi (2013) carried out embryo sexing using sperms selected by modified bovine serum albumin gradient technique in rams. They found that percentage of male: female embryos ranged from 54.5 – 72.7%.

Percoll gradients centrifugation can be used in sperm preparation for *in vitro* fertilization (Mohammed et al., 2005) and can be omitted for intracytoplasmic sperm injection. Percoll and its contemporaries separate motile from non-motile sperm and impact on sperm capacitation. Biological basis for using the gradient preparation is to reduce the generation of ROS oxygen radicals, selects the best fraction of motile and normal sperms. Moreover, there is no adverse effect on fertilization and embryo cleavage. Polyvinylpyrrolidon - coated silica particles (Percoll gradients) were withdrawn from markets in 1996 because of the risk of contamination with endotoxins (Svalander *et al.,* 1995).

1.3. H-Y antigen:

H-Y antigen expression on sperm bearing Y-chromosome has been reported in some species and human as well. The H-Y antigen expression has a slightly higher frequency in human sperm bearing Y-chromosome, but its expression among sperm bearing X-chromosome is also considerable (Sills et al., 1998). Techniques of immunology relying on this antigen are unlikely to effect the sex selection of human sperm. Hendriksen et al. (1993) did not yield evidence that H-Y antigen is preferentially expressed in sperm bearing Y-chromosome.

1.4. Electrophoretic separation:

Electrophoretic sperm separation based on sperm size and electronegative charge has been reported. The suspensions of electrophoretic separation technique contained normal spermatozoa. This procedure of sperm separation is both time and cost-effective. The first pregnancy using electrophoretic sperm separation was reported for a couple suffering from extensive sperm DNA damage (Ainsworth et al., 2007). Fertilization of oocytes using IVF and ICSI procedures with sperm electrophretically prepared gave comparable results concerning fertilization rates, cleavage and embryo quality (Fleming et al., 2008).

1.5. Flow cytometry:

Flow cytometry is the most reliable method for separating X- and Y-bearing sperm but it requires expensive equipments (Flaherty et al., 1997). This technique routinely separates fractions of spermatozoa with a purity greater than 80%. Flow cytometry technique was used to sort human spermatozoa firstly by Dr. Glenn Spaulding during the early to mid-1980s. Semen is labeled with a Hoechst 33342 fluorescent dye prior to flow cytometric sorting, which binds to the DNA of spermatozoon. Fluorescence released from each sperm was detected through a 400 nm long pass filter, and then the sorted sample was collected. X-chromosome has more DNA than Y-chromosome; human 2.9; cattle 3.8; chinchilla 7.5 turkey 0. The female spermatozoa bearing X-chromosome will absorb a greater amount of dye than spermatozoa bearing Y-chromosome because the X-chromosome has more DNA than the Y-chromosome. The separation of sperm population is dependent on the species. Purities of spermatozoa of cattle and sheep for each sex will usually remain above 90% depending on 'gating', while for human the purities may be reduced to 90% for female spermatozoa and 70% for male spermatozoa. Drawbacks of flow sperm cytometry is very costly, decread viability of spermatozoa, less number of spermatoza sorted per hour ($3.5 \text{ X } 10^5$).

2. Methods of embryo sexing:

First successful embryo sexing done by Gardner in 1968 in rabbits by cytological method (Barr body). Embryo sexing is determined in pre-implantation embryos. Thereafter, splitting of sexed embryos might be applied and transferred to recepient animals. There are various methods of embryo sexing have been applied. Embryo sexing were carried out for cattle, buffalo, sheep, goats, horses and pigs, (Hirayama et al., 2013) and human as well (Mori and Shiota, 1994). Single cell is sufficient for determination of embryo sexing (Chrenek et al., 2001). Non electrophoretic method for PCR sexing reduced time requirement to less than 2 hours (Hasler et al., 2002). There are various methods for embryo sexing divided to non invasive and invasive methods (figure 2).

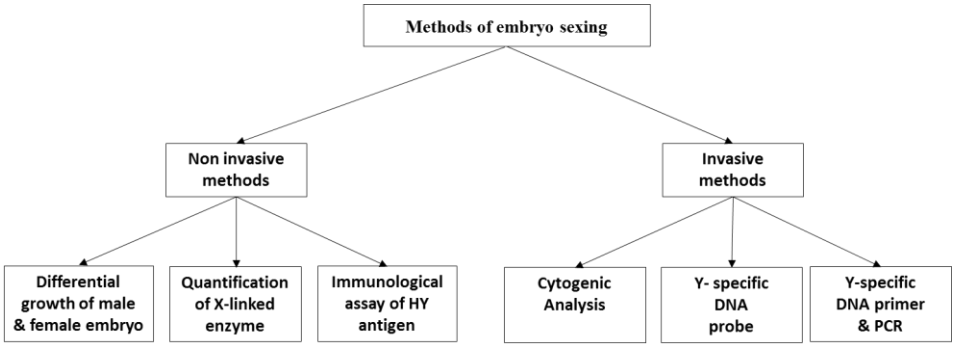


Fig. 2: Methods of embryo sexing

2.1. Non invasive methods:

2.1.1. Differential growth of male & female embryo (Yadav et al., 1992):

The hypothesis is that male embryos develop more rapidly than female ones (Dumoulin et al., 2005). Sex-related growth differences in preimplantation embryos was found in some animal species. Fanchin *et al.* (1998) found similar growth rates of female and male human embryos before the eight-cell stage. Dumoulin et al. (2005) analysed cell numbers of male and female human embryos that developed to the blastocyst stage after either in vitro (IVF) or intracytoplasmic sperm injection (ICSI) for investigating sex-dependent growth rates. They found that the sex-related growth difference of embryos is sex dependent after fertilization using ICSI technique but not after IVF method.

2.1.2. Quantification of X-linked enzyme:

Quantification of X-linked enzyme has been reported as method of embryo sexing (Williams, 1986). X-linked enzyme assay as glucose 6-phosphate dehydrogenase (G6PD) allowed the prediction of embryo sexing without biopsy of blastomeres (Williams, 1986) and the accuracy was 64%. This technique was based on the findings that female embryos have two X-chromosomes that seem to be potentially active in early embryos (Epstein, 1969). Iwata et al. (2002) found that activity of G6PD enzyme was high in female morula compared with male one.

2.1.3. Immunological assay of H-Y antigen:

Antibodies of immunological method target male and female embryos. This method is non invasive and rapid method. The accuracy of this method is 98%. The antibody is protein in Y-shaped found on the surface of B cells and is released into the blood or lymph in response to an antigenic stimulus. Male and female sex specific proteins (SSP) are obtained from membrane of donor tissue. Antibodies are prepared to respond to SSP of both sexes. Antibodies targeting desired-sex cells are tagged with fluorescent molecules. Tagged antibodies are introduced into embryos that require sexing. Antibodies bind to target receptors on the embryos. Embryos of same sex as antibody will appear green under fluorescent light. H-Y detection was used for sexing murine, bovine, porcine embryos and the detection was as ealy as 8 cell stage embryos. H-Y antigen was detected in swine embryos only after removal of zona pellucida. Accuracy of H-Y detection was 84% in cattle, 85% in goat, 81% in pig, 88% in sheep (White et al., 1987). Poor quality embryos show fluorescence unrelated to presence of antigen. Reproducibility and reliability of these noninvasive methods were insufficient for practical use.

2.2. Invasive methods:

2.2.1.Cytogentic analysis:

A Barr body is the inactive X-chromosome in a female somatic cell (Lyon, 2003). Cytogenetic analysis in cattle was tried to identify sex of trophoblast biopsies initially from day 12 to 15 embryos with accuracy 58.5-68% (Hare et al., 1976), bisected embryos (Seike et al., 1990) and biopsies from day 6 to 7 embryos (Singh and Hare, 1980). The limiting factor with the cytogenetic analysis technique of embryo sexing is the relative number of cells in metaphase. The time required to process 12-15 embryos is 5 hours.

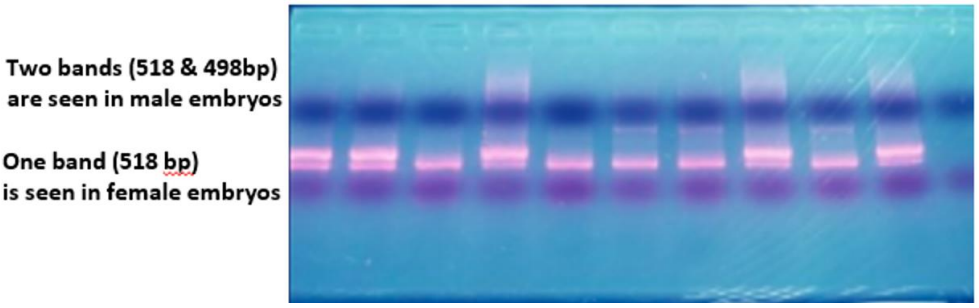
2.2.2.Y- Specific DNA probe:

Identification sequences of Y- specific DNA enabled to develop molecular technique for embryo sexing of different species. Fluorescence *in situ* hybridization (FISH) for Y-chromosome-specific DNA sequences was used to distinguish between male and female cells (Cotinot et al., 1991; Cenariu et al., 2011). An accurate and rapid method using FISH has been reported (Kobayashi et al., 2004). The accuracy of the FISH method of bovine embryo sexing was of 86.66% (Cenariu et al., 2011).

2.2.3. Embryo sexing through PCR:

Molecular techniques have been used to differentiate the sex of embryos. Rapid sexing within 2 hours by using multiplex polymerase chain reaction (PCR) has been recorded (Cenariu *et al.,* 2011). Blastomere is aspirated form excellent or good embryos at stage of compact morula or blastocyst and used to obtain DNA. PCR assay was carried out on DNA. If the embryo is male, two bands are seen (518 and 498 bp) whereas the female embryo 518 bp band is only seen (figure 3) (http://www.atuttascuola.it/tecno/cavenati/test_dna.htm). Embryo sexing through PCR has been reported in several species as mice (Bradbury *et al.,* 1990), pig (Pomp et al. (1995), sheep (Saravanan et al., 2002) and human as well (Handyside et al., 1989). The accuracy of the PCR method of bovine embryo sexing was of 96.4% (Cenariu *et al.,* 2011).

The PCR sexing of bovine embryos yielded better results than the FISH method. Polymerase chain reaction (PCR) offered invaluable advantage of being so fast in comparison with the earlier methods. This advantage enabled to transfer embryos to female recipient without cryopreservation. However, PCR is not an easy technology for embryo sexing in the field due to the required strict thermal control for primer annealing and DNA synthesis and electrophoresis to visualize amplified products in addition to the risk of false positives because of DNA contamination. Therefore, for the wide use of embryo sexing in embryo transfer industry of mammals, a simple, rapid and precise embryo sexing method needs to be developed.



Conclusions:

Preselection of embryo sexing at the time of conception is necessitate in some cases in animals and humans as well. Progress has been achieved in preselection of embryo sexing at the time of conception in spite of constraints but it is still very expensive and time consuming. With the advancement of technology, it becomes precise, cheap and more affordable. Embryo sexing has medical and agriculture application through gender balancing, sex linked disorders, decreased number of culled animals and cost. The application extends to cloning, transgenic, sports and racing animals. The overall goal of embryo sexing is decreasing the cost by limiting number of animals required to produce the products.

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