**Embryo Sexing and it’s future perspective in Livestock Farming**

Arindam Bhowmik

Department of Veterinary Medicine

College of Veterninary Sciences and Animal Husbandry,

Central Agricultural University, Selesih, Aizawl, Mizoram-796015

**Introduction**

A technique called embryo sexing is used to determine an embryo's gender prior to implantation. This method of managing animals is effective. This embryo sexing method is more effective in dairy farming because female calves are always preferred in the dairy sector. Both female and male cattle produce milk and meat, which is advantageous for the dairy and beef businesses (Sachan et al., 2020). In breeding programs, embryo sexing is particularly beneficial for enhancing management and productivity. Additionally, it might aid in the early detection of genetic abnormalities. Pre-implantation sexing of embryos improves embryo transfer effectiveness and makes it easier to transfer the desired embryos based on their sex (Bredbacka, 2001; Cenariu et al., 2008). In vitro fertilization (IVF), or artificial insemination, has been used in recent years to achieve desired sex, however it is expensive (Seidel Jr, 2007) and less effective than traditional unsorted semen (Trigal et al., 2012). In heterosexual twins, the sex prediction of embryos before to implantation also aids in preventing freemartins. The producers can swiftly grow their herd by running fewer recipient females thanks to embryo sexing. Pre-selection of female progeny through embryo sexing is crucial for preserving endangered species. In the near future, the sexing of embryos is likely to be used widely in the embryo transfer sector. Importance of pre-determining of sex in any animal breeding strategy through embryo transfer allowing procedures to concentrate their genetic improvement on their male or female lines through better utilization of recipient females.

**Different techniques of Embryo sexing**

The type of spermatozoon chromosomes that fertilized the ovum determines the zygote's sex. The spermatozoon's X and Y chromosomes determine whether a zygote is male or female, respectively. Both invasive and non-invasive methods of embryo sexing are available, depending on the patient's needs. Non-invasive techniques are preferred since the embryos' integrity and viability are preserved (Utsumi and Iritani, 1993).

Non-invasive techniques can measure the embryo's normal growth, but their precision for identifying the embryo's sex is limited (Sharma et al. 2017). A Y-chromosome-specific probe is a more accurate method to determine the sex of an embryo than intrusive procedures, which are less significant because there is a danger of damaging the embryo. The sex of an embryo can now be determined using new molecular techniques, such as polymerase chain reaction (PCR) and fluorescent in situ hybridization (FISH), which are quicker and more accurate.

Methods of embryo sexing can be categorized as:

**I. Invasive methods**

A. Cytological method or Karyotyping

B. Identification of sex chromatin

C. Y chromosome specific DNA probes

D. Polymerase chain reaction (PCR)

E. Loop mediated isothermal amplifications (LAMP)

F. Fluorescence *in situ* hybridization (FISH)

**II. Non–invasive method**

The embryo is not subjected to any harm throughout the procedure

A. Detection of X-linked enzymes

B. Detection H-Y antigens

C. Sexing based on cleavage and development

**I. Invasive methods**

**Cytological method or Karyotyping**

Karyotyping is done using cells at metaphase stage of mitosis when cells divisions are being stopped by culturing with colchicines.Then the cells are allowed to swell to disperse the chromosome. Following fixation and staining with a permanent DNA dye, such as Giemsa, the slides are examined under a microscope. Cells, which are arrested in metaphase, generate a spread of chromosomes that can be identified by their specific banding patterns. The Y-chromosome is easily identified by its small size. The accuracy of sexing by using this method is nearly always 100% (Seidel, 1999).

**Advantages**:

* High accuracy rate.
* Less requirement of sophisticated equipments
* Inexpensive and easy to perform
* It can identify chromosomal abnormalities before the embryos are transferred.

**Disadvantages**

* Intensive labor and time consuming process..
* Chances of accidental harm to survived chromosomes.
* Reduce the viability and conception rate of embryos

**B. Identification of sex chromatin**

Identification of sex chromatin depends on the presence of “Barr body”, a dark stained moiety, near to the nucleus in a cell. Barr body formation results from inactivation one of the X chromosome present in female cell not from male cells. In 1949, Barr and Bertram identified the condensed inactive X chromosome or Barr body in female nucleus. Demonstration of sex of embryo by evaluating sex chromatin of Rabbit trophoblastic cells was performed by Edward and Gardner (1958).

**Advantages:** This is a simple and rapid technique

**Disadvantages:**

* Barr body may not present in all cells.
* Granular cyctoplasm sometime prevents the detection of Barr body.
* This method is not suitable for cattle, sheep, goat, pig and horse due to coarse nature of the chromatin (Betteridge *et al*., 1982).

**C. Y chromosome specific DNA probes**

This technique is one of the most accurate methods of determining the male sex embryo by presence of Y chromosome. In this method small quantity cells of embryo are collected by using micro or biopsy blade with proteinases to expose the DNA then hybridized with radioactively labeled Y-chromosome specific probe. By using biotinylated Y-chromosome specific probe, sexing of bovine embryo can be identified within 30 minutes (Leonard *et al*., 1987).

**Advantages**

* High accuracy and a higher percentage of embryos can be sexed.
* Sexing can be done from a little quantity of DNS sample.
* DNA can be used in Fluorescence in situ hybridization (FISH) to distinguish between male and female cells (Cotinot *et al*.,1991)

**Disadvantages**

* Quite expensive, complicated and time consuming process.
* Collection of biopsy material from embryo is not accessible all the times. (Vliet *et al*.,1989).

**D. Polymerase chain reaction (PCR)**

As of right now (Da Cruz et al. 2012), PCR is the method of choice for predicting fetal sex using DNA fragment from maternal plasma. The Y chromosome's particular DNA sequence may be amplified by PCR, which works well for sexing bovine embryos. Embryo sexing by PCR entails a biopsy of the embryo (1-4 blastomeres), amplification of two DNA fragments (one species- and one male-specific), and interpretation following electrophoresis analysis of the amplified products. Aasen and Medrano (1990) conducted the first sexing of a goat embryo using PCR amplified DNA from a blood sample. Goat sex can be determined using very accurate PCR sexing techniques based on the amelogenin gene (Chen *et al*., 2007).

**Advantage**

* It is a sensible, accurate and reliable method.
* Less damage to embryo while collection of sample as very little quantity is needed for PCR.
* It can be used for genotyping and testing of genetic diseases as well.

**Disadvantages**

* It requires high technical knowledge and skill.
* Time consuming process.
* It may give false positive result due to contamination of DNA.

**E. Loop mediated isothermal amplifications (LAMP)**

Loop mediated isothermal amplification (LAMP) is a DNA amplification method that can amplify a specific DNA sequence within the range of 60 to 650C. Field application of LAMP based embryo sexing has been attempted (Hirayam *et al*., 2004). Here amplification products can be detected via photometry turbidity caused by magnesium pyrophosphate intercalating dyes. LAMP can amplify a target sequence within about 15 minutes. Accuracy and sensitivity of LAMP based sexing of bovine embryo is very high and reliable.

**Advantages**

* Rapid, sensitive method for field application.
* Like PCR LAMP does not need electrophoresis to detect amplified DND products.
* Less chances of damage of embryonic tissues.

**Disadvantages:** Expensive and high technical knowledge requires operating the technique

**F. Fluorescence *in situ* hybridization (FISH)**

The technique fluorescence *in situ* hybridisation (FISH) can detect specific DNA sequences of individual chromosomes from a cell (Kobayashi *et al.* 2004). This method can be used not only to predict the sex of embryo but also detect the chromosomal mosaicism and aneuploidy in embryos (Griffin *et al.* 1992; Delhanty *et al.* 1993). Unlike the PCR, chances of contamination of sample are very less in FISH (Sharma *et al.* 2017). Male and female embryos can be differentiated by using DNA probe specific to Y chromosome in fluorescence *in situ* hybridization (FISH) (Cotinot *et al.* 1991). Cenariu *et al*. (2011) reported the accuracy of the FISH method of bovine embryo sexing is 86.66%.

**Advantages:**  It is highly sensitive and accurate embryo sexing technique compared to PCA.

**Disadvantage:**  It is complicated, expensive and time consuming method.

**II. Non–invasive method**

**A. Detection of X-linked enzymes**

The female has two X chromosomes whereas the male has only one X chromosomes in somatic cells and hence the enzymes associates with X chromosomes produced in female are almost double than that produced in the males. These enzymes are Glucose-6- phosphate dehydrogenase (G6PD), Hypoxanthine guanine phosphoribosyl transferase (HPRT), Phosphoglycerate kinase, Agalactosidase. These enzymes are measured in embryos. The high concentration of enzyme usually denotes two X chromosomes or female embryo. While low concentration denotes one X chromosomes or male embryo. (Monk and Handyside, 1988). In bovine embryos, glucose and glutamine metabolism has been studied by Tiffin *et al*. (1991) and showed greater metabolism of glucose and glutamine in female embryos than male embryos.

**Advantages**

* Allowing all embryos to be sexed.
* Accuracy is almost 90 percent for female and 100 percent for males sex

**Disadvantage**

* Estimation has to be done for very small quantity enzymes.
* Chances of false diagnosis due to variations.
* This test may toxic to embryo.

**B. Detection H-Y antigens**

An additional non-invasive approach to sexing embryos is provided by the immunological demonstration of a sex-specific antigen. The H-Y antigen can be found in embryos using either an immunofluorescent technique or a cytotoxicity assay. Embryos are exposed to diluted H-Y antiserum and complement in the cytotoxicity experiment. Male embryos are defined as those that express the H-Y antigen and have some degree of cell lysis. The immunofluorescent assay system sometimes wrongly referred to as the anti-H-Y antigen method requires antibodies to cell-surface chemicals specific to male organs. Embryos are incubated for 30-60 minutes with antibodies, and then for an additional 30-60 minutes with an antibody to the first antibody containing fluorescin isothiocyanate (FITC), a fluorescent dye. Then, an embryo is briefly viewed under a fluorescent microscope. The embryos of males glow. It has been demonstrated that all mammalian species, including mice, rats, pigs, sheep, goats, cows, and horses, express it in pre implantation embryos. The accuracy of the H-Y antigen assays in determining the sex of an embryo is about 85% (Anderson, 1987).

**Advantages:** It is a rapid test and no need of biopsy of embryo.

**Disadvantages:** Lengthy process of embryo may reduce the conception rate and unavailability of fluorescence microscope.

**C. Sexing based on cleavage and development**

Amount of DNS is less in male cells of embryo compared to female embryo cells. Hence it takes a longer cells cycle and time to duplicate. The male embryos are considered to cleave early and develop fast to attain morula and blastocyst stage than female embryos. As per some recent reports cleavage and development is faster in male embryo than female embryo in both *in vivo* and *in vitro* (Sharma *et al.* 2017).

**Advantages:** Accuracy and sensitivity is very high

**Disadvantages:**

* Need high skills.
* Cleavage time cannot be predicted.
* May decrease the embryo viability.

**Conclusion and future possibilities**

There are several techniques for sexing embryos, each with pros and cons. For sexing embryos, Polymerase Chain Reaction (PCR), LAMP, and FISH are the most user-friendly, effective, and accurate procedures available. Predetermination of sex in a Multiple Ovulation Embryo Transfer (MOET) nucleus breeding strategy could either raise the female-to-male ratio, improving selection accuracy, or decrease the number of males generated, decreasing MOET costs. Additionally, embryo sexing aids in zoo breeding practices and endangered wildlife species conservation. Cloning and the generation of transgenic animals both benefit from it. More research needs to be done to accurately predict the embryonic sex based on the differences between the development of male and female embryos. On the basis of the distinct hormone profiles of fetuses of each sex, the sexing of the embryo may be possible. The content of estrogens and androgens in blastocoels can be used to predict embryonic sexing in pig and horse embryos (Sharma et al. 2017). According to Larson et al. (2001), there are some sex-specific mechanisms in bovine embryos that control the signaling processes of implantation. They claimed that female embryos produce more signaling factors, such as interferon tau. Similar to female embryos, male embryos develop more quickly in vitro when exposed to higher serum glucose concentrations (Bredbacka and Bredbacka, 1996; Gutierrez-Adan *et al*., 2001). All of these techniques still need to be explored further in order to increase their efficacy and accuracy. For commercial reasons, the livestock industry has to use embryo sexing procedures, and as more effective technologies become available, demand for these techniques is expected to rise.

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