

EMBRYO SEXING AND ITS FUTURE PERSPECTIVE IN LIVESTOCK FARMING

Abstract

This chapter describes the different techniques of embryo sexing and its application in livestock farming. Embryo sexing is a technique for determining the sex of embryo in its early state before implantation. This embryo sexing method is more effective in dairy farming because female calves are always preferred in the dairy sector. In breeding programs, embryo sexing is particularly beneficial for enhancing management and productivity. Additionally, it might aid in the early detection of genetic abnormalities. Embryo sexing is especially helpful for improving management and production in breeding projects. The sex prediction of embryos before implantation helps to prevent freemartins in heterosexual twins. The preservation of endangered species depends on the pre-selection of female offspring through embryo sexing. Pre-determining sex in any animal breeding strategy through embryo transfer is essential since it enables processes to focus on improving the genetics of their male or female lines through better utilization of recipient females. There are different methods to determine the gender of the fetus, each of which has advantages and disadvantages. Polymerase chain reaction (PCR), LAMP, and FISH are among the easiest, most effective, and most accurate methods available to determine the sex of the fetus. All these techniques need further research to improve their efficiency and accuracy. Commercial reasons require the livestock industry to use fetal sex determination methods, and demand for these techniques is expected to increase as more effective techniques become available.

Keywords: Embryo Sexing, Livestock Farming, Fetus, Effective Techniques

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I. INTRODUCTION

A technique called embryo sexing is used to determine an embryo's gender before 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. Embryo sexing is especially helpful for improving management and production in breeding projects. It might also help with the early diagnosis of genetic anomalies. Pre-implantation embryo sexing increases the efficiency of embryo transfer and makes it simpler to transfer the desired embryos based on their sex (Bredbacka, 2001; Cenariu et al., 2008). Artificial insemination, also known as in vitro fertilization (IVF), has been applied in recent years to produce desired sex, but it is more expensive (Seidel Jr., 2007) and less successful than conventional unsorted semen (Trigal et al., 2012). The sex prediction of embryos before to implantation helps to prevent freemartins in heterosexual twins. As a result of embryo sexing, producers can quickly increase their herd size by running fewer recipient females. The preservation of endangered species depends on the pre-selection of female offspring through embryo sexing. The sexing of embryos will probably become an established procedure in the embryo transfer industry soon. Pre-determining sex in any animal breeding strategy through embryo transfer is essential since it enables processes to focus on improving the genetics of their male or female lines through better utilization of recipient females.

II. DIFFERENT TECHNIQUES OF EMBRYO SEXING

The zygote's sex is determined by the type of spermatozoon chromosomes that fertilized the ovum. A zygote's gender is determined by the X and Y chromosomes of the spermatozoon, respectively. Depending on the patient's demands, both invasive and non-invasive methods of embryo sexing are available. Since the integrity and viability of the embryos are protected, non-invasive procedures are preferable (Utsumi and Iritani, 1993).

Normal embryo growth can be measured using non-invasive methods, however their precision for determining the embryo's sex is restricted (Sharma et al. 2017). Instead of invasive methods, which are less important because there is a risk of harming the embryo, a Y-chromosome-specific probe is a more precise way to identify the sex of an embryo. New molecular methods, such as the speedier and more precise polymerase chain reaction (PCR) and fluorescent in situ hybridization (FISH), can now be used to identify the sex of an embryo.

Methods of embryo sexing can be categorized as

1. Invasive Methods

- Cytological method or Karyotyping
- Identification of sex chromatin
- Y chromosome-specific DNA probes
- Polymerase chain reaction (PCR)

- Loop-mediated isothermal amplification (LAMP)
- Fluorescence in situ hybridization (FISH)

2. Non-Invasive Method: The embryo is not subjected to any harm throughout the procedure

- Detection of X-linked enzymes
- Detection of H-Y antigens
- Sexing based on cleavage and development

III. INVASIVE METHODS

1. Cytological method or Karyotyping: Karyotype analysis is performed using cells in mid-mitosis, where cell division has been arrested by incubation with colchicine. The cells then expand to disperse the chromosomes. After fixation and staining with a permanent DNA dye such as Giemsa, the slides are examined under a microscope. Cells that are arrested in metaphase produce chromosome spreads that can be identified by distinct banding patterns. The Y chromosome is easily identified by its small size. Sex accuracy using this method is almost always 100% (Seidel, 1999).

- **Advantages**

- High accuracy rate.
- Less requirement for sophisticated equipment
- Inexpensive and easy to perform
- It can identify chromosomal abnormalities before the embryos are transferred.

- **Disadvantages**

- Intensive labour and a time-consuming process.
- Chances of accidental harm to survived chromosomes.
- Reduce the viability and conception rate of embryos

2. Identification of Sex Chromatin: Identification of sex chromatin depends on the presence of "cargo bodies", darkly colored regions near the nucleus within the cell. Barr body formation occurs when one of the X chromosomes, which is present in female cells but not in male cells, is inactivated. In 1949, Barr and Bertram identified an inactive X chromosome, or Barr body, condensed in the female nucleus. Demonstration of embryonic sex by evaluation of sex chromatin in rabbit trophoblast cells was done by Edward and Gardner (1958).

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

- **Disadvantages**

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

3. Y Chromosome-Specific DNA Probes: This method is one of the most accurate methods of determining male embryos with the presence of Y chromosome. This method uses a proteinase-equipped micro or biopsy blade to collect a small amount of cells from the embryo, exposes the DNA, and radiolabels it with a Y-chromosome-specific probe. Using a biotinylated Y-chromosome-specific probe, the sex of bovine fetuses can be determined 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 small quantity of DNS samples.
- DNA can be used in Fluorescence in situ hybridization (FISH) to distinguish between male and female cells (Cotinot et al.,1991)

- **Disadvantages**

- Quite an expensive, complicated and time-consuming process.
- The collection of biopsy material from embryos is not accessible all the time. (Vliet et al.,1989).

4. Polymerase Chain Reaction (PCR): Currently (Da Cruz et al. 2012), PCR is the method of choice for fetal sex prediction using DNA fragments from maternal plasma. Specific DNA sequences on the Y chromosome can be amplified by PCR, which works well for sex determination in bovine embryos. Determining the gender of the embryo by PCR includes biopsy of the embryo (1 to 4 blastomeres), amplification of two DNA fragments (one species-specific and the other male-specific) and electrophoretic analysis of the amplified products. It needs interpretation. Aasen and Medrano (1990) performed the first sex determination of goat fetuses using PCR-amplified DNA from blood samples. Goat sex can be determined using a highly accurate PCR sexing technique based on the amelogenin gene (Chen et al., 2007).

- **Advantage**

- It is a sensible, accurate and reliable method.
- Less damage to the embryo while collecting samples 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 results due to contamination of DNA.

5. Loop-Mediated Isothermal Amplification (LAMP): Loop-mediated isothermal amplification (LAMP) is a DNA amplification method that can amplify specific DNA sequences in the temperature range of 60-65°C. Field applications of LAMP-based embryo sex determination have been attempted (Hirayam et al., 2004). Here, the amplification products can be detected through the photometric turbidity caused by the internal colors of magnesium pyrophosphate. LAMP can amplify target sequences in about 15 minutes. The accuracy and sensitivity of determining the sex of the cow embryo based on LAMP is very high and reliable.

- **Advantages**
 - A rapid, sensitive method for field application.
 - Like PCR LAMP does not need electrophoresis to detect amplified DND products.
 - Less chances of damage to embryonic tissues.
- **Disadvantages:** Expensive and high technical knowledge requires operating the technique

6. Fluorescence in situ hybridization (FISH): Fluorescence in situ hybridization (FISH) techniques can detect specific DNA sequences on individual chromosomes from cells (Kobayashi et al. 2004). This method can be used not only to predict fetal sex, but also to detect chromosomal mosaicism and fetal aneuploidy (Griffin et al., 1992; Delhanty et al., 1993). Unlike PCR, FISH has very little chance of sample contamination (Sharma et al. 2017). Male and female embryos can be distinguished by fluorescence in situ hybridization (FISH) using a DNA probe specific for the Y chromosome (Cotinot et al. 1991). (2011) reported that the accuracy of the FISH method for sex discrimination in bovine embryos was 86.66%.

- **Advantages:** It is a highly sensitive and accurate embryo sexing technique compared to PCA.
- **Disadvantage:** It is a complicated, expensive and time-consuming method.

IV. NON-INVASIVE METHOD

1. Detection of X-Linked Enzymes: Females have two X chromosomes in their somatic cells, but males only have one X chromosome in their somatic cells, so the X chromosome-related enzymes produced in females are produced in males almost twice as much. These enzymes are glucose-6-phosphate dehydrogenase (G6PD), hypoxanthine guanine phosphoribosyl transferase (HPRT), phosphoglycerate kinase and agalactosidase. These enzymes are measured in the fetus. A high concentration of the enzyme usually indicates two X chromosomes or a female foetus. On the other hand, low concentrations indicate an X chromosome or a male foetus. (Monk and Handyside, 1988). In the bovine embryo, the metabolism of glucose and glutamine has been studied by Tiffin et al. (1991) who showed that female fetuses have better glucose and glutamine metabolism than male fetuses.

- **Advantages**
 - Allowing all embryos to be sexed.
 - Accuracy is almost 90 per cent for females and 100 per cent for males sex
- **Disadvantage**
 - Estimation has to be done for very small quantities of enzymes.
 - Chances of false diagnosis due to variations.
 - This test may be toxic to the embryo.

2. Detection of H-Y Antigens: Immunological demonstration of sex-specific antigens provides an additional non-invasive approach to fetal gender identification. H-Y antigen can be found in the fetus using immunofluorescence or cytotoxicity method. For cytotoxicity experiments, embryos are exposed to antiserum and diluted H-Y complement. Male embryos are defined as embryos that express the H-Y antigen and have some degree of cell lysis. The immunofluorescence assay system, sometimes erroneously called the anti-H-Y antigen assay, requires antibodies to specific cell surface chemicals for the male organ. Embryos are incubated with antibodies for 30-60 minutes and then with primary antibody antibodies containing the fluorescent dye fluorescein isothiocyanate (FITC) for another 30-60 minutes. The embryos are then briefly observed under a fluorescence microscope. The male fetus shines. It has been shown to be expressed in preimplantation embryos by all mammalian species, including mouse, rat, pig, sheep, goat, cow, and horse. The accuracy of the H-Y antigen assay in determining the sex of the fetus is approximately 85% (Anderson, 1987).

- **Advantages:** It is a rapid test and no need for a biopsy of the embryo.
- **Disadvantages:** The lengthy process of the embryo may reduce the conception rate and the unavailability of a fluorescence microscope.

3. Sexing based on Cleavage and Development: The amount of DNS is less in male cells of the embryo compared to female embryo cells. Hence it takes a longer cell cycle and time to duplicate. The male embryos are considered to cleave early and develop faster to attain the 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 embryo viability.

V. CONCLUSION AND FUTURE POSSIBILITIES

There are different methods to determine the gender of the fetus, each of which has advantages and disadvantages. Polymerase chain reaction (PCR), LAMP and FISH are among the easiest, most effective and most accurate methods available to determine the sex of the fetus. Predetermined sexing in multiple oviposition embryo transfer (MOET) nuclear breeding strategies may increase the sex ratio and improve selection accuracy or reduce the number of males produced to reduce MOET costs. Additionally, embryo sex determination is useful for zoo breeding practices and conservation of endangered wildlife species. Both cloning and production of transgenic animals benefit from it. More research should be done to accurately predict fetal gender based on developmental differences between male and female fetuses. Based on the different hormonal profiles of the fetuses of each sex, it may be possible to determine the gender of the fetus. Estrogen and androgen content in the blastodermal cavity can be used to predict the embryonic sex of pig and horse embryos

(Sharma et al. 2017). According to Larsson et al. (2001), several sex-specific mechanisms control the implantation signaling process in the bovine embryo. They reasoned that female embryos produce more signaling factors such as tau interferon. Similar to female embryos, male embryos grow faster in vitro when exposed to higher serum glucose concentrations (Bredbacka and Bredbacka, 1996; Gutierrez-Adan et al., 2001). All these techniques need further research to improve their efficiency and accuracy. Commercial reasons require the livestock industry to use fetal sex determination methods, and demand for these techniques is expected to increase as more effective techniques become available.

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