Sexing embryo

Introduction

For sexing embryo, two methods are followed and they are non-invasive and invasive method depending on whether not a biopsy of embryonic tissue is required [2]. Criteria that must be considered in embryo sexing techniques are the percentage of embryos that can be accurately sexed and the effect that the sexing procedure may have on embryo viability [1].

A. Non-invasive method

A non-invasive approach is considered to be optimal because it maintains the integrity of the embryo and so is less likely to impair the potential for successful embryo transfer and implantation. Under non- invasive approach, there are two techniques, one is monitoring of x- linked enzyme activity prior to x chromosome inactivation and second is immunoreactions with embryos with antibodies to a sex-specific antigen [4].

1. X-linked enzymes: In normal mammals, homogametic sex (female) carries two X chromosomes (XX) whereas heterogametic sex (male) possesses only one X chromosome (XY). In female, one of the X chromosome is in activated in each cell in embryonic life to maintain an equivalent number of genes between sexes [5]. The exact timing of X-inactivation is not known, but studies suggested that there is a brief period between activation of the embryonic genome and X-inactivation in which genes from both X chromosome in the female are transcribed. This is reflected in cellular concentration and activity of certain X linked enzymes that is twice as high in female as in male embryos. But in both cases, X- linked enzyme activity must be compared to autosomal enzyme activity to account for individual variation in embryo metabolism [4]. Ultimately, the ratio of X-linked enzyme activity to autosomal enzyme activity will be higher in female than in male embryos. Williams (1986) demonstrated that morula to blastocyst staged embryo can be examined for activity of a X- linked enzyme namely glucose 6-phosphate dehydrogenase (G6PD) to determine the sex of embryo. Monk and Handyside (1988) revealed that X-linked hypoxanthine phosphoribosyl transferase (HPRT) and autosomal adenine phosphoribosyl transferase (APRT) activity could be measured and compared to evaluate the sex of embryo [4]. But,this technique has certain limitations like:

- There are high mortality rates of embryos during quantification of enzymes through staining because of toxicity arisen from extensive enzyme activity and retention of the stain.
- The exact period of x-inactivation is not known in most of the domestic species.
- Activation of embryonic genome occur as late as 8-cell stage in mice and 8- to 16-cell stage in cow, hence care must be taken in such assays to ensure that the enzymes monitored are the result of embryonic transcription and not derived from the translation of stored maternal mRNA [8].

2. H-Y antigen: H-Y antigen is an histocompatibility antigen found on the surface of male but not on female cells. It was first recognized in a closely inbred strain of mice when the females rejected male but not the female skin grafts. An antiserum to H-Y antigen can be produced in the inbred strain of mice by inoculating female animals with male cells over a period of time and this anti-serum can be used for detection of H-Y antigen [1]. Two methods exist for detecting H-Y antigen on embryos such a cytotoxicity assay and an immunofluorescent assay.

- **Cytotoxicity assay**-In cytotoxicity assay, embryos are exposed to H-Y antiserum and complement. Embryos expressing H-Y antigen show a degree of cell lysis and thus, are categorised as male. In this method, embryo survival rate is low which is the most disadvantage of this procedure [9].
- **Immunofluorescent assay**-It involves exposing embryos to primary H-Y antibody for 30 minutes, followed by reaction with a secondary antibody to which fluorescein isothiocyanate (FITC) has been conjugated. Embryos are then evaluated for the presence or absence of the FITC tag under a fluorescent microscope [6].

The major limitation of these two immunological sexing assays is that the accuracy of sexing does not appear to exceed 90%because, H-Y antigen is a relatively weak antigen to detect

through antigen-antibody reaction, secondary antibodies occasionally show nonspecific -binding, H-Y antigen may not be limited exclusively to male embryos and finally, subjectivity is involved in judging the degree of fluorescence of the antibody tag [9].

B. Invasive method

A number of invasive methods are practically being used by the breeders for efficient sexing of embryos and have been briefed below.

1. Cytogenetic analysis: Cytogenetic sexing was the first method to produce sexed rabbits, calves and sheep. This technique requires the biopsy of a small number of cells which are cultured in a medium with colchicines (a mitosis arresting agent), cells are introduced to swell so that chromosomes are dispersed, slides are prepared, fixed and stained with permanent DNA dye like Giemsa and finally, slides are examined under microscope. All the chromosomes are viewed and X as well as Y chromosome are easily identified by its tiny size [7].

In this assay, detection of two X chromosome or one Y chromosome is considered sufficient indication of sex interpreting two X chromosome in female and Y chromosome in male embryo [3]. The accuracy of sexing using this method is nearly 100%. The limitations of this method are the difficulties in obtaining biopsies which show high quality metaphase spreads of chromosomes, requirement of trained cytogeneticists and this method is time consuming [1].

2. Y-specific DNA probes: This technique involves biopsy of a small number of cells from embryo and hybridization of total cellular DNA to a labelled sequence of DNA (probe) that is specific to Y chromosome. Such labelling of probe can be done by radioactive method or non-radioactive method like fluorescent *in situ* hybridization [5]. Positive hybridization results indicate the presence of Y chromosome and thus the male sex of embryo. This method is very attractive because it needs small amount of materials from embryo for DNA preparation and this method also detect sex by 100% accuracy [3].

3. PCR based method: This technique requires biopsy of blastomeres from preimplantation embryo for sexing. In this method, Y-chromosome based DNA sequence is used to design the primer and amplification [5]. The nested primer extension preamplification- and multiplex PCR are known to be efficient ways to amplify the low DNA template and to detect Y-specific DNA fragment through the gel electrophoresis [7].

4. Quantitative realtime PCR:-This method involves biopsy of embryo, isolation of DNA and realtime PCR with Y-chromosome specific DNA based primers and Taqman probes [8]. Finally, the presence and quantification of Y-chromosome specific DNA fragments has been possible to detect accurate sex of embryos. This method is highly efficient and needs very small amount of embryonic cells [7].

Conclusion

Sexing of embryo is very much essential to increase the efficiency of livestock production. It is mainly done by two methods which includes invasive and non-invasive method. Non-invasive method is comparatively better than invasive method. Because, the embryos which are sexed using invasive method, cannot be better in efficient implantation when compared to embryos which are handled non-invasively. But the accuracy of sex determination will be more or it is almost 100% with the embryos sexed using invasive method rather than non-invasive method. Hence, the sexing of embryos either by invasive or non-invasive method mainly depends on the need for embryo sexing.