EMBRYO SEXING- RECENT APPROACHES IN DOMESTIC ANIMALS

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INTRODUCTION

The desire to manipulate sex is for obvious in Livestock industry. Some sectors of the livestock industry benefit from a higher proportion of male progeny, the most notable being beef stud breeders. In most cases however female progeny are preferred for milk production and their reproductive potential. In countries like India where cattle slaughter is banned, the male calves are just a burden for the livestock industry and lowering the economy of the livestock farmer. It would be most efficient to control the sex at the time of conception so all offspring would be of the desired sex. The development of embryo transfer procedures has created the potential for embryo sexing as an alternative method of controlling the sex of offspring. Several approaches have been used to determine the sex of bovine embryos prior to embryo transfer .some of them are being discussed below.

1.Detection of H-YAntigen

The male specific H-YAntigen has been found on the surface of somatic cells in the heterogametic sex of all the species. Using immunological techniques, the presence of H-YAntigen has been demonstrated on cells of 8-cell stage through to the blastocyst stage in bovine embryos and after this stage it becomes increasingly difficult to detect. When embryos are incubated *in vitro* with this antisera in the presence of compliment, lysis of male cell results (embryo cytotoxicity assay). Embryo cytotoxicity assays as described above is limited to the production of female embryos only as male embryos will be destroyed. This limitation can be overcome by using fluorescent tagged antisera and avoiding compliment in the incubating media. The advantages of an immunological approach to embryo sexing are considerable. This procedure is noninvasive and require no specific manipulation skills and embryo viability apparently is not compromised.

2.Cytogenetics analysis or Karyotyping.

The presence of Barr body (examined by Aceto orcien staining) formed from the inactive X-chromosome in female cells is not reliable indicator of sex in case of domestic animals. The granular nature of the cytoplasm makes observation of the barrbody difficult, and some female embryos may be determined to be males due to the absence of barr body before X chromosome inactivation is complete. Blastocyst stage of roughly 6-8 days post conception/IVF in bovine are cultured with a mitosis arresting agent (like colchicines). The cell nuclei are expanded in a hypnotic salt solution, and individual sex chromosome and autosomes are examined microscopically. The requirement for the skilled cytogeneticist and the inefficiencies of technique make it unlikely that the cytogenetics approach to embryo sexing will have commercial application. However it may be used to confirm the results achieved by alternative methods of embryo sexing.

3. X- Linked enzyme analysis

The mammalian males are XY and females has XX sex chromosome compliment. The exact timing of the X-Chromosome inactivation in embryos of domestic animals is not known, but it likely begins to occur during the blastocyst stage. For a brief time during the embryonic development before X chromosome inactivation, embryos theoretically can be distinguished as male or female by measurement of gene dosage for X-linked enzymes. The cellular concentration and activity of certain enzymes including Glucose -6- phosphate dehydrogenase (G6PD), hypoxanthine phosphoribosyl transferase (HPRT) have been investigated. The approach of embryo sexing is complicated by the fact that variable X chromosome dosage is limited to the period after the activation of embryonic genome and before X-chromosome inactivation. Moreover reports suggest the assay may be toxic to the embryos.

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4. Y specific DNA Probe

Several patented Y specific DNA probes have been made available for sheep cattle and man. This method is applied to individual embryos. Embryo biopsy is the first step for this and which can be done by many microsurgical procedures like aspiration or by micro blade biopsy. Transfer of biopsy to the PCR tube is critical step in embryo sexing technique. PCR amplification of the Y- Chromosome specific gene(SRY) and an autosomal gene (Aml-X) as internal control is the second step in embryo sexing. On electrophoresis the presence of Y-Chromosomal fragments indicate male and its absence indicates female sample, while autosomal fragments present in both samples. Because in this procedure both the fragments are co-amplifies, the PCR is known as Duplex-PCR. PCR Probing is undoubtedly the most accurate method of sexing embryos, but more widespread commercial applications are limited by the fact that embryos have to be probed individually , necessitating skilful micromanipulation.

5. LAMP (Loop Mediated isothermal amplifications)-

LAMP is simple and easy to perform technique which is both sensitive and rapid under isothermal conditions. The product of LAMP is detected by the turbidity of a reaction mixture without electrophoresis. The LAMP reaction includes autocycling, strand displacement DNA synthesis. A specially designed set of two inner and outer primers is used, but later during the cycling reaction only the inner primers are used for strand displacement DNA synthesis. So it amplifies target sequences with high selectivity. One of the characteristics of LAMP is its ability to synthesize an extremely large amount of DNA. Accordingly large amount of byproduct magnesium pyrophosphate is produced in the form of white precipitate, which allows easy detection of nucleic acids amplified by the LAMP method. The total time needed for embryo sexing is 1 hour and accuracy of sexing is 75-100%. No special apparatus is needed, which makes it more economical and practical than nested PCR and Real time PCR. Sex determination with this technique has been found to be more sensitive than polymerase chain reaction. LAMP based embryo sexing has been found to determine gender accurately and it is suitable for field application

6. Differential development rates.

Studies reported that developmental rates of embryos are sex dependent, and that male embryos develop at a faster rate than female embryos. Sex dependent developmental rates have also been reported for *in vitro* fertilized embryos. Avery et al.,1991 reported that 95% of fastest developing embryos were male. Practical significance of this approach is poor due to the relative inability to predict the sex of the entire population of embryos.

Commercial Applications

Routine use of embryo sexing is economically encouraged by the savings in recipient procurement and maintenance if embryos of the undesired sex are not transferred. Because commercial embryo transfer is restricted to the purebred industry, individual offspring of the desired sex are of high value and any loss of production of these individuals carries a high cost. The costs associated with international shipment of embryos (health tests, duties, etc.) make it highly advantageous to ship only embryos of the desired sex. The only sex determination procedures (Y-specific probes) that have been used at a commercial level are invasive and result in damage to the zona pellucida. Because of a concern for disease transmission from embryos without an intact zona pellucida , no country, at this time, will accept for import embryos with a damaged zona pellucida from embryo sexing procedures. With present technology, embryo sexing based on Y-chromo- some-specific sequences is the method of choice because of its high degree of accuracy.

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