THE USE OF AMNIOCENTESIS IN PRENATAL DIAGNOSIS OF OVINE (Ovis aries) SEX

(UTILIZAÇÃO DA AMNIOCÊNTESE NO DIAGNÓSTICO PRÉ-NATAL EM OVINOS (Ovis aries))

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SUMMARY

The objective of this study was to analyze by cytogenetic analysis ovine fetal amniotic cells by amniocentesis for sex determination and later elimination of animals with abnormal chromosomal abnormalities. Ten ml of amniotic fluid was punctured from females at three different stages of pregnancy (76, 108, and 145 days), and 55 samples from 37 fetuses were cultured. Samples from 8 fetuses were successfully cultured. Conventional culture techniques and CBG banding were used for sex determination. One hundred percent of the analyzed samples were correctly sexed. The recommended pregnancy stage for material collection was around 108 days, approximately 2/3 of pregnancy. The major limitation of this technique lies in amniotic cell culture. Thus, alternative procedures to improve cell culture will have to be tested to make it a routine procedure in veterinary medicine.


INTRODUCTION

RIIS & FUCHS (1960) first used amniocentesis for the detection of fetal sex in man. Since then, the procedures for amniocentesis and the methods for amniotic cell cultures in vitro have been improved. Today, the detection of fetal sex and antenatal diagnosis of chromosomal disorders are important aspects of genetic counseling and obstetric practices in human medicine.

In veterinary medicine, however, amniocentesis...
has not been widely used, except for bovine sex determination. BONGSO & BASRUR (1975) reported that prenatal genetic analysis was not only possible, but it was also accurate in sex diagnosis of 17 male bovine fetuses. SINGH & HARE (1977), analyzing 42 bovine amniotic cells reported appropriate growth in 29 samples. Twenty-four of these samples showed correctly sexed fetuses, two incorrectly sexed, and three although sexed, could not be confirmed. LEIBO & RALL (1990) diagnosed the sex in 1,056 bovine fetuses in embryo transfer programs. The objective of these authors was to use amniocentesis routinely to diagnose sex of bovine fetuses produced by embryo transfer, and then alter the sex ratio of the population.

KAMIMURA et al., (1997) and MAKONDO et al., (1998) reported on the use of amniocentesis in fetal sex determination using polymerase chain reaction (PCR) for DNA amplification of fetal cells and later hybridization, using probes allele-specific of genes linked to sex chromosomes. SHEA (1999) used PCR for bovine sex determination, but in his experiment, the DNA was obtained from embryo biopsies. This author concluded that the pregnancy rate obtained after embryo sexing was appropriate for commercial purposes.

In addition to these experiments on prenatal sex determination, only a few studies were performed attempting to elucidate the extent of embryonic chromosomal disorders as the primary cause of embryo mortality. HARE et al., (1980) and GAYERIE (1984) published papers on bovine, while LONG & WILLIAMS (1980) and MURRAY et al., (1985), focused on ovine. LONG & WILLIAMS (1980) studied 89 cells from embryos and non-fertilized eggs collected 2 to 3 days after mating. These authors found a 6% incidence of chromosomal abnormalities, indicating the existence of chromosomally abnormal embryos in the first few days of pregnancy. MURRAY et al., (1985) analyzed the chromosomes of 73 cells from 177 Merino embryos at an early stage of cell division. Chromosomal aberrations were seen in 11% of the embryos. Polyspermy was considered as one of the main causes of abnormal embryos.

The objective of this study was to cytoogenetically analyze ovine fetus amniotic cells for sex determination and later elimination of animals with abnormal, possibly compromising chromosomal analysis.

MATERIAL AND METHODS

The amniotic fluid was punctured from 64 pregnant females at three different stages of pregnancy, around 76, 108, and 145 days. The animals belonged to the Veterinary Hospital of the School of Veterinary Medicine and Animal Husbandry, Botucatu – UNESP. The amniotic fluid was taken to the laboratory in a sterile syringe at room temperature.

Culture and analysis of amniotic cells

The amniotic fluid was centrifuged at 1,500 RPM for 30 minutes. The cells were resuspended and transferred to sterile culture flasks containing 3 ml of Chang’s medium (# T105 – Irvine Scientific), 20% of bovine fetal serum (Gibco), and 0.1 ml of penicillin/streptomycin (Gibco). This material was incubated between 10 to 30 days in a 5% CO2 incubator at 38º C. Cells were harvested adding 0.1 ml of 0.016% colchicine mitogen (Sigma) and incubated for 45 min at 38º C. The culture flask medium was transferred to a centrifuge tube and added 2 ml of buffered trypsin (ATV – Instituto Adolfo Lutz), and incubated for 3 to 6 min at 38º C. All this material was replaced in the same centrifuge tube and 6 ml of Hanks’ saline solution (Instituto Adolfo Lutz) was added to the culture flask for the last washing. This material was then transferred to another centrifuge tube, centrifuged at 1,500 RPM for 5 min, and 5 ml of 0.8% sodium citrate saline was added for 20 min at 38º C. This material was centrifuged again at 1,500 RPM for 5 min, fixed, and washed twice in 5 ml of a cold solution consisting of 3:1 mixture of methanol-glacial acetic acid. The final pellet was resuspended in 0.5 ml fixative and the slides were prepared. For conventional analysis, the slides were stained for 5 min in 5% Giemsa. CBG banding was obtained using the modified technique as described by SUMMER (1972).

Analysis method

Ten cells from each animal were analyzed using conventional staining and CBG banding. After observing that the Y chromosome was present in all metaphases, the fetus was diagnosed as male. In the sample showing 2 X chromosomes in one metaphase of 56 chromosomes, 25 cells were analyzed. From these, if no male cells were found, the fetus was diagnosed as female. However, if at least one male cell was found, 25 additional metaphases were analyzed to confirm diagnosis.

RESULTS

Fifty-five samples of amniotic cells from 37 fetuses were cultured, with successful results in 8 cultured samples. Figures 1, 2 and 3 show the amniotic cell growth and fixation 10 days after culture. After analysis using conventional staining with Giemsa, which revealed 56 chromosomes, CBG banding was used as an aid in the
Figure 1 – Amniotic cell growth and fixation 10 days after culture in a 5% CO₂ incubator at 38°C, using an inverted microscope with 10x magnification. The arrows show fusiform cells, which are beginning to adhere to the culture flask.

Figure 2 – Amniotic cell growth and fixation 10 days after culture in a 5% CO₂ incubator at 38°C, using an inverted microscope with 20x magnification. The arrows show dendrite-shaped cells attached to the flask.

prenatal diagnosis of fetal sex. Figures 4 and 5 show mitotic metaphases from male and female fetuses with CBG banding staining.

DISCUSSION

After the cesarean section and sex confirmation, it was found that all the samples analyzed using tissue culture were correctly sexed. This result shows that prenatal cytogenetic analysis is not only possible but also reliable. Abortion as a side effect of this procedure was observed in only one animal after the first collection, which represents 2% of the total number of pregnancies. The risk of abortion as well as induced congenital malformations may be kept to a minimum, as long as the fluid collection and fetus manipulation are appropriately performed.

The recommended stage of pregnancy for collection, where there is a higher availability of cells for culture, was around 108 days, approximately 2/3 of pregnancy. LEIBO & RALL (1990) concluded that the culture time for bovine amniotic cells to reach a concentration sufficient for analysis was around 13 days for samples collected between 7 and 8 weeks and 8 and 10 days for those collected between 10 and 15 weeks or more, which corresponds to 2/3 of pregnancy. It is important to keep in mind that the cells harvested from human amniotic fluid are obtained only from fetuses (CASADEI et al., 1973). However, in both bovine (JORDAN & KINDRED, 1970) and ovine the fluid can be obtained either from the amniotic or the chorion-allantoic cavities. In this study,
Figure 3 – Growth and fixation of amniotic cells 10 days after culture in a 5% CO₂ incubator at 38º C, using an inverted microscope with 40x magnification. The continuous arrow shows a nucleus during interphase, with a rounded outline and a golden edge. This is characteristic of cells that will start dividing, that is, undergoing intense metabolic activity. The arrow indicates the place where cytokinesis will occur. The dashed arrow shows a divided cell attempting to adhere.

Figure 4 – Mitotic metaphase obtained from the culture of a male fetus’s amniotic cells, using CBG banding. The arrows indicate the X chromosomes.

Figure 5 – Mitotic metaphase obtained from the culture of a female fetus’s amniotic cells, using CBG banding. The arrows indicate the X chromosomes.

The allantoic fluid collected during the cesarean section was not appropriate for culture. The proportion of allantoic and amniotic fluids changes during the pregnancy’s trimesters; the allantoic is in excess during the first and third trimesters of pregnancy (ARTHUR, 1969).

The major limitations of this technique were related to amniotic cell cultures. Thus, alternative procedures will have to be tested to improve culture and make it a routine procedure in veterinary medicine. Sex determination and posterior selection could decrease the number of individuals of the same sex evaluated over a certain period of time, increasing selective intensity and reducing costs of ovine embryo transfer.

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REFERENCES


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