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Research Article

Chorionic Villus Sampling (CVS) in Cows

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Abstract: Eleven slaughtered Holstein and 11 slaughtered Holstein x Anatolian black pregnant cows (10 female, 12 male fetuses) were studied. Gestational ages of the cattle ranged from 67 days to 245 days. The average ages for the first trimester and second trimester were 79.58 ± 3.04 and 113.89 ± 4.08 days, respectively. In this first attempt, 3 cultures failed and no metaphase could be obtained. In the rest of the cases 9 females with 60 XX and 10 males with 60 XY were obtained. In addition to normal metaphase plates, a high incidence of tetraploid plates belonging to normal fetus phenotypes was observed. There was no correlation between the gestational age and the number of metaphases obtained. No numerical or structural abnormality was observed in the chromosomes of the study group. The detection of the genetic sex of cattle is relatively easy, since medium-sized submetacentric chromosomes are XX and the small submetasentric chromosome is Y, and the rest of the autosomal chromosomes are acrocentric.

Key Words: Chorionic villus sampling, cow, sex determination, chromosome abnormality

İneklerde Chorionic Villus Sampling (CVS)

Özet: Çalışmada materyal olarak, kesime sevk edilmiş gebe 11 Holstein ve 11 Holstein X Anadolu Esmeri inek (10 dişi, 12 erkek fötus) kullanıldı. İneklerin gebelik dönemleri 67 ile 245 gün arasında değişmekteydi. Gebeliğin ilk ve ikinci 1/3'lük dönemlerindeki gebelik süresi sırasıyla 79,58 ± 3,04 ve 113,89 ± 4,08 gün olarak belirlendi. İlk aşamada üç kültürde başarı sağlanamadı ve metafaz aşaması gözlenemedi. Geri kalan olgularda, 60XX'li 9 dişi ve 60XY'li 10 erkek karyotip elde edildi. Normal metafaz plaklarına ek olarak normal fötus fenotiplerine ait tetraploid plakların insidensi de yüksek olarak gözlendi. Gebelik süresi ile metafaz sayısı arasında korelasyon görülmedi. Çalışma grubunun kromozomlarında sayısal veya yapısal anormallik gözlenmedi. Ineklerde genetik olarak cinsiyetin belirlenmesi kolaydır çünkü 2 orta büyüklükteki submetasentrik kromozomlar XX ve küçük submetasentrik kromozomlar XY olup, geri kalan tüm kromozomlar akrosentriktir.

Anahtar Sözcükler: Chorionic villus sampling, inek, cinsiyet tayini, kromozom anomalileri

Introduction

Recent developments in reproductive and cellular biology, and cytogenetics offer prospects for the emergence of a number of methodologies that might be useful in animal breeding (1). Among these, cytogenetic analysis to determine chromosomal abnormalities and sex differentiation for the evaluation of X-linked diseases in the fetal period is used worldwide in humans (2,3). On the other hand, sex determination in the cattle industry has enabled breeders to change the projection of the management in animal breeding before parturition. Different sexes are used in different production branches in cattle breeding, and determination of the sex ratio could lead to increased production. For that reason, the sexing of cattles in the early period is economically effective and useful. For example, it was recently reported that the fertility reducing effects of translocation led to a loss of approximately $250,000 (4).

On the other hand, from the medical point of view, it was estimated that 2% of all late gestation abortuses and stillbirths may have chromosomal abnormalities (5). Early determination of chromosomal abnormalities will provide an opportunity for early pregnancy termination and reduce the time for a later pregnancy.

Cytogenetic studies in cattle have been performed on peripheral blood (6,7), amniotic fluid (8), fibroblasts (5),

bone marrow (9) and lymph nodes (6) and especially blastocysts are preferred for sex determination (1,10); however, chorionic villus sampling (CVS) is not used commonly. In fact, prenatal sexing using CVS can be performed earlier than amniocentesis, and can be obtained by a transabdominal or transcervical approach (11-13). Several studies have suggested that CVS has a 0.5-1.0% greater risk than amniocentesis of causing miscarriage in cattle (11,13). Nevertheless, in the event of detection of any chromosomal defect or unwanted sex for economic reasons, abortion can be induced with minimal hazard to subsequent fertility (14).

The purpose of this study was to perform fetal sexing and to analyze the chromosomes of cattle conceptuses by direct CVS.

Materials and Methods

Cows

Eleven Holstein and 11 Holstein x Anatolian black pregnant cows (10 female, 12 male fetuses) were included in the study. Gestational ages of the cattle ranged from 67 days to 245 days. Among these, 12 cows were in the first trimester, 9 in the second trimester and 1 in the third trimester. The average ages of cows in the first trimester and second trimester were 79.58 ± 3.04 and 113.89 ± 4.08 days, respectively.

Obtaining Specimens

Placentas obtained from a slaughterhouse were brought to the laboratory in an icebox. They were examined and their chorionic villi were separated under the inverted microscopy in the laboratory. Chorionic villi were directly put in sterile test tubes containing RPMI 1640 medium, and transferred to the laboratory immediately. The direct method of obtaining chromosomes from CVS was applied to the samples (11,12,14).

Cytogenetic Analysis

The RPMI 1640 medium (Biological Industries, Cat. No: 01-100-1B, Kibbutz Beit Haemek, Israel), supplemented with 25% fetal calf serum (Seromed, Cat. No: D 12247, Berlin, Germany), and penicillin and streptomycin (Biological Industries, Cat. No: 03-031-1C, Kibbutz Beit Haemek, Israel) antibiotics were used. The contents of the test tubes were transferred to 35-mm plastic petri dishes. The chorionic villi were separated completely from the maternal decidua and blood, and they were minced carefully. The samples were transferred to new sterile petri dishes, each of them containing 3 ml of medium. A 0.1 mg/ml final concentration of colcemid (Biological Industries Cat No: 12-003-1C, Kibbutz Beit Haemek, Israel) was added to the dishes and they were incubated at 37 ºC for 3 h. At the end of this period, the culture medium was removed with a micropipette and the villi were treated with hypotonic solution (1% sodium citrate) for 20 min at 37 ºC.

The hypotonic solution was replaced by cold, freshly prepared Carnoy's fixative (3:1 methanol:acetic acid) and the cells were fixed at room temperature for 20 min. Then the fixative was removed and a second fixative was added, followed by storage at –20 ºC in a deep freeze overnight. The next day, the villi were treated with a third fixative, again for 20 min. and finally the samples were placed in 0.5 ml of 60% glacial acetic acid for 5-10 min. and the suspension was transferred to slides heated at 60 ºC on a hot plate and spread by an L-shaped Pasteur pipette. The slides were air dried and stained with Giemsa. Some of the preparations were G banded (14).

Metaphases were counted and analyzed by Olympus CH 30 microscope (10x100). Some photographs taken at metaphase are depicted in the Figure.

Results

The mean crown-rump lengths (CRLs) of embryos belonging to the first, second, and third trimesters were 10.96 ± 1.21, 24.72 ± 1.64 and 77 mm, respectively (Table).

In this first attempt, 3 cultures failed and no metaphase could be obtained, giving a success rate of 86.3%. In the rest of the cases 9 females with 60 XX and 10 males with 60 XY were obtained (Figure).

In addition to normal metaphase plates, a high incidence of tetraploid plates belonging to normal fetus phenotypes was also observed. There was no correlation between the gestational age and the number of metaphases obtained. No numerical or structural abnormality was observed in the chromosomes of the study group. The detection of the genetic sex of cattle is relatively easy, since 2 medium-sized submetacentric chromosomes are XX and the small submetasentric chromosome is Y, and the rest of the autosomal

Table : Cytogenetic results of the cattle with different gestational ages.

Sample no.	CRL (mm)	Fetus age (day)	Trimester	Karyotype
1	19.0	100	2	60,XY
2	27.5	121	2	60,XX
3	77.0	245	3	60,XX
4	24.0	112	2	60,XX
5	12.0	82	1	60,XY
6	28.0	123	2	60,XX
7	11.5	81	1	60,XY
8	24.5	113	2	60,XX
9	34.0	137	2	60,XY
10	22.0	107	2	60,XX
11	8.5	73	1	60,XY
12	11.5	81	1	60,XY
13	7.0	70	1	60,XX
14	6.0	67	1	60,XY
15	15.0	90	1	_
16	12.0	82	1	_
17	12.0	82	1	60,XX
18	18.0	97	2	60,XX
19	7.0	70	1	60,XY
20	8.0	72	1	60,XY
21	25.0	115	2	60,XY
22	21.0	105	1	_

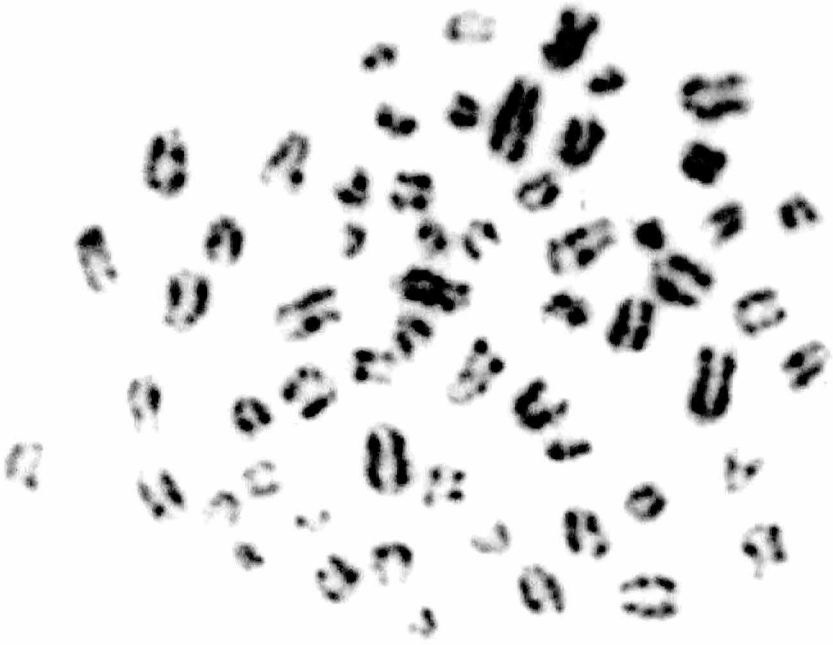

Figure. Metaphase plate of cattle obtained by CVS.

chromosomes are acrocentric. Similarly, it was also quite easy to identify the sex chromosomes and determine the sex of the fetus in our study.

Discussion

Since many anomalies have been correlated with such problems as embryonic death, abortion, congenital abnormalities and reduced reproductive efficiency in mammalian species, it is important to use cytogenetic analysis in addition to other laboratory techniques (15). Cattle cytogenetics started with the detection of 1/29 translocation in 1964 and this investigation not only had a major impact on cattle cytogenetics but also fostered the discipline in other domestic species (14). The well-documented anomalies for cattle are trisomy 17, trisomy 18, sex chromosome rearrangements and translocations (especially 1/29 Robersonian translocation) (16), insertion 16 (17), paracentric inversions (18), chromatid and chromosome breaks (19), transposition of nucleolar organizing regions (14) and XX/XY chimerism (15,20). The widespread distribution in cattle of the 1/29 Robertsonian translocation and its effect of reducing fertility by increased embryonic mortality in both male and female heterozygotes has been reported (16). The diagnosis of cytogenetic defects in the fetus such as translocations and pericentric inversions and abnormal sex chromosome constitution (15) causing low fertility may be used as the basis for deciding the further course of action with regard to the early elimination of such calves by induced abortion (2,16). Other accidents of fertilization may produce point mutations, replications and invasions (16), especially affecting the economics of cattle production (21). In our cases we did not observe such karyotypes. Compared with humans, relatively few data are available on the incidence of numerical and structural chromosomal anomalies in domestic animals, although chromosomal abnormalities are known to affect the animal breeding industry. One reason for this is the technical difficulty in obtaining good quality metaphase. In our study, similar problems were observed and the appearance of tetraploidy at a high rate could be explained by the rapid division of cells in that gestational period such as observed in pigs, or may be due to the effect of long duration colcemid treatment in harvesting (Personal communication with Popescu). A very similar observation was reported in another study showing that the tetraploid ratio in the peripheral blood lymphocyte cultures in Romanian cattle breeds was as high as 20%, and had no phenotypic effect. This was considered due to the culture conditions and not to genetics (20).

Although obtaining poor quality or no metaphase chromosomes has been reported (3), and similar difficulties were observed in our studies, this could represent species specific chromosomal fixation differences.

The early detection of fetal sex in cattle by chromosome analysis using direct CVS may contribute to better planning, management and establishment of a dairy or beef herd in a relatively short time. CVS could also help to reduce some inherited chromosome rearrangement, which causes sex chromosome aberrations (4). The most frequently reported sex chromosome anomalies in cattle were XXX, XXY and XYY. However, we did not observe any sex chromosome anomaly in our study.

CVS can be performed by a transabdominal or transcervical approach in women, and about 4% of them had a miscarriage due to CVS (22). We suggest that CVS be performed by transrectal ultrasonographic or laparo-hysteroscopic routes in cows. However, as this test has not been performed in cows for continuing pregnancy, data for the risk of miscarriage could not be obtained.

We conclude that direct CVS could be feasibly used for the cytogenetic analysis of cattle chromosomes, giving the opportunity to determine the sex of the fetus as early as possible, such as at 67 days of gestation, and to determine the structural or chromosomal abnormalities in cattle.

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