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Sex Determination of Fetus Prenatal from Maternal Plasma in Goats Using Duplex PCR

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Abstract

This work was carried out the Genetic Engineering Lab, College of Agriculture, Basrah University, Iraq. This study included determines the sex of the fetus prenatal by analyzing free fetal DNA cells in maternal plasma by using duplex PCR. blood were collected from 5 pregnant females prepared the plasma and extract the DNA by using heat-based direct method for plasma and then investigate the sequences GAPDH and DYS14 through the use of multiplex polymerase chain reaction technique and the results were 2 (40%) males show a double bands one at 97 bp representing GAPDH and the second at 198 bp representing DYS14 and 3 (60%) females show a single band at 97 bp representing GAPDH.

Keywords: Goat maternal plasma; Duplex PCR; Sex determination

Introduction

There is a simple and effective method for noninvasively detecting fetal sex using circulation fetal DNA from first trimester maternal plasma. Fetal sex determination is important in some endocrine diseases, such as congenital adrenal hyperplasia, where there is masculinization of the female fetus, which is preventable with antenatal treatment. Generally, early fetal sex determination has been performed by invasive procedures, such as chorionic villus sampling or amniocentesis. However, these invasive procedures still carry a 1 to 2% risk of miscarriage [1,2] and cannot be performed until 11 week of gestation. Moreover, reliable determination of fetal sex using ultrasonography cannot be performed in the first trimester, because the development of external genitalia is not complete [3]. Circulating fetal DNA arises from trophoblasts in the placenta and is released directly into the circulation system of the mother [4,5]. It represents 3-6% of the total cell-free DNA that is present in maternal circulation and is rapidly cleared from the maternal circulation after delivery [6,7]. Therefore, circulating fetal DNA has been proposed as a potential material for noninvasive prenatal diagnosis (NIPD). Many researchers have explored the possibility of circulating fetal DNA on NIPD of fetal sex, RhD blood typing, and single-gene disorders, such as cystic fibrosis [8,9]. Promising results have been reported from studies using the combination of markers such as Detection Y-Specific gene (DYS14) presence of this gene indicates a male fetus and glyceraldehyde-3-phosphate dehydrogenase(GAPDH) sequence found in both sex [8,9]. Lo et al. (1998) demonstrated that free fetal DNA (ffDNA) circulating in maternal blood has a relatively high concentration in early pregnancy, which increases during gestation and degrades in a few hours after delivery [7,10,11]. Detection of ffDNA in maternal blood during pregnancy has given rise to the possibility of developing new noninvasive approaches for early prenatal diagnosis [12]. This approach seem to be more useful in human as compared with animals because it enable the diagnosis of the fetal gender in early gestation period this led to eliminate the risk of some disease such as Mendelian diseases, also prediction of RhD genotype and in addition to fetal gender [13,12].

Materials and Methods

Analysis of cell-free fetal DNA from maternal plasma using a multiplex PCR

Peripheral blood samples obtained from 5 healthy goat males were used to determine the PCR specificity and sensitivity, whereas peripheral blood samples obtained from 5 non pregnant goat female served as negative controls and also from 5 pregnant goat females use to determine the gender of fetus at agricultural research station in agriculture college, university of Basrah.

Plasma preparation

Plasma in this study was prepared according to [14]. For plasma preparation, collect 2-3 ml into an EDTA-containing tube for plasma separation .The blood samples were centrifuged at 3000 rpm for 10 min and the plasma was carefully removed from their tubes and transferred into plain polypropylene tubes. The plasma samples then submitted a second centrifugation at 3000 rpm for 10 min and these centrifuged plasma samples were transferred into fresh polypropylene tubes and the samples were stored at -20°C until DNA extraction.

DNA extraction from plasma using the heat-based direct method

The DNA extracted from plasma using the heat-based direct method according to [15].

200 µl of plasma in a 0.5 µl sterile Eppendorf tube was heated at 99°C for 5 min.

The heated sample was then centrifuged at 12000 rpm for 10 minutes in a cooling micro-centrifuge.

After which the clear supernatant was collected and 10 µl used for PCR reaction.

Measurement quantity of extracted DNA from plasma maternal using Nanodrop technique

The DNA quantity is measured by using Nanodrop technique (Thermo Scientific company/USA) as in (Figure 1) to detect genomic size (DNA quantity ng/µl).

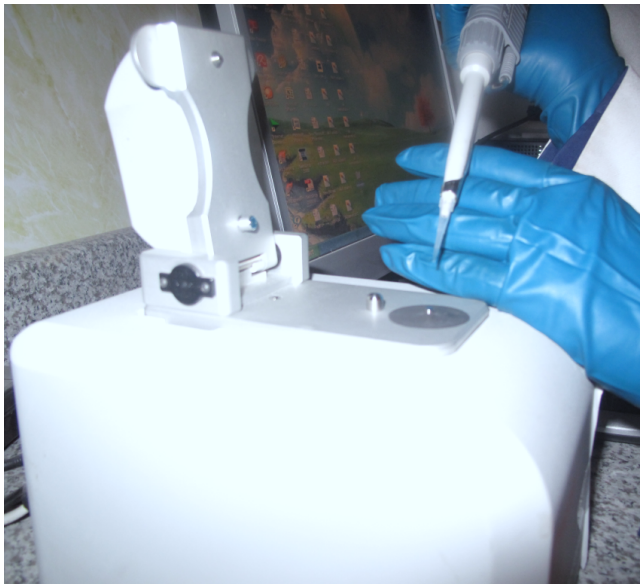

Figure 1: Showing measurement DNA quantity ng/µl using Nanodrop technique.

Amplification of DYS14 gene and GAPDH using duplex PCR

The DYS14 and GAPDH genes were studied according to protocol of [16]. DYS14 and GAPDH genes were amplified in a duplex PCR setting for the detection of fetal DNA in maternal plasma. The PCR amplification mixture showed in Table 1-3.

Chemical material	Master Mix	Primers	DNA Template	Nuclease free water	Final volume
Volume	25	2.5 of each forward and reverse primer	10	5	50

Table 1: Showing PCR product composition (µl).

PCR result analysis

The results of PCR were performed after the amplification process. 5 µl from amplified sample was directly loaded in a 2% agarose gel that prepare containing 0.5 µl/25 ml ethidium bromide with the addition of loading buffer and DNA size marker as standard in electrophoresis and run at 80 V for 40 minutes then the products were visualized by gel documentation system (S 140, Votornix, USA).

Primer name	Description and size of primer	sequence (5→3)	PCR product size (bp)
Y1.7	(21)	5'-CATCCAGAGCGTCCCTGGCTT-3'	198
Y1.8	(21)	5'-CTTTCCACAGCCACATTTGTC-3'	
GAPDH	Forward primer (21)	5'-CCCCACACACATGCACTTACC-3'	97
	Reverse primer (21)	5'-CCTAGTCCCAGGGCTTTGATT-3'	

Table 2: Oligonucleotide primers sequences used for a duplex PCR amplification of DYS14 and GAPDH genes according to Lale et al. [16].

Step	Temperature (°C)	Time (Min)	No. of cycles
Initial denaturation	95	15	1
Denaturation	94	1	40
Annealing	57	1	
Extension	72	1	
Final extension	72	10	1

Table 3: A duplex PCR amplification program for DYS14 and GAPDH genes detection according to Lale et al. [16]

Results

Detection of DYS14 and GAPDH by using duplex PCR for gender diagnosis

The PCR analysis was applied for DNA extracted from plasma by heat-based direct method which characterized by using the extracted DNA directly in PCR reaction because when the extracted DNA delayed to use will not give results when amplification and the PCR reaction were failed. After DNA extraction method, the quantity of DNA measured by Nanodrop technique (Figures 2 and 3). The PCR were positive for both DYS14 and GAPDH sequences in DNA samples from all 5 healthy males. While in DNA samples extracted from non-pregnant females the PCR were negative for the DYS14 sequence, but positive for GAPDH sequence in all 5 non pregnant females. The results of gender diagnosis were 2 (40%) male and 3 (60%) females as show in (Table 4) from the DNA samples was extracted from 5 pregnant females (Figure 4).

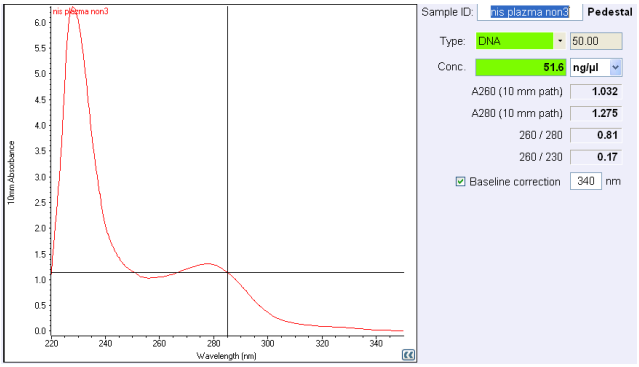

Figure 2: Measurement quantity of DNA (ng/µl) from non-pregnant female plasma using Nanodrop technique.

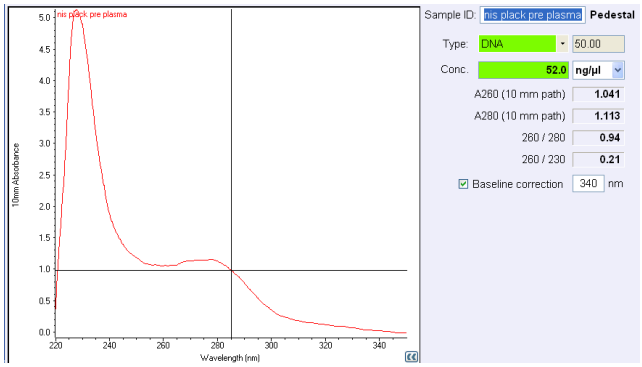

Figure 3: Measurement quantity of DNA (ng/µl) from pregnant females-plasma using Nanodrop technique.

No. of pregnant goat females	Amplification of DYS14	Amplification of GAPDH	Gender detected
1	+	+	Male
2	+	+	Male
3	_	+	Female
4	_	+	Female
5	_	+	Female

Table 4: Results of amplification of DYS14 and GAPDH using duplex PCR for gender diagnosis from maternal plasma in pregnant females.

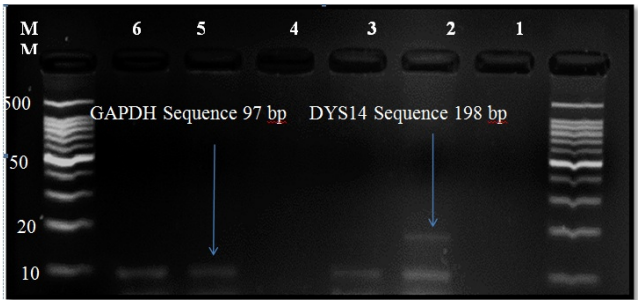

Figure 4: Showing duplex PCR setup for DYS14 (198 bp) and GAPDH (97 bp) electrophoresis in 2% agarose. M Lane=DNA ladder, Lane 2=male (has two bands one at 198 bp (DYS14) and other at 97 bp (GAPDH), Lane 3, 5, 6=female, has one band at 97 bp (GAPDH).

Discussion

This study also used to diagnosis the gender of embryo from pregnant female circulation this done by extraction the DNA from maternal plasma by using the heat-based direct method, this method characterized by using the extracted DNA directly in PCR reaction [15]. At the first amplified the DNA extracted from male and non-pregnant female goat plasma which used as control to confirm the accuracy of this technique to detect the gender and the results were accordance with actual sex. In this study the technique applied on 5 pregnant goats female to detect the gender. The results were 2 (40%) male and 3 (60%) female. When the embryo are male the result of amplified were two bands, one 198 bp represent the DYS14 sequence, and other 97 bp represent the GAPDH sequence, while when embryo are female the results of PCR are one band at 97 bp represent GAPDH sequence. These results agreed with many investigators [14,16-18].

Conclusions

Sex determination of fetus prenatal by analysis of ffDNA in maternal plasma is reliable technique but need more sterilized condition to avoid the mistake results.

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