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Identification of Pseudohermaphrodite Polled Apparently Female Goats Based on *ZFX* and *SRY* Sex Determination

Research Article

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ABSTRACT

The presence of intersexuality in goat breeds is one of the most popular, and challenging issues. Therefore, understanding the genetic complexity of this phenomenon at the DNA level may help to discover the molecular mechanisms of sexual differentiation in this species. A solution to this problem is proposed in the development of a DNA test using sex-determination candidate genes. With this motivation, the main goal of the present report was to identify the pseudohermaphrodite polled goats based on *ZFX* and *SRY* sex-determination candidate gene findings. Overall, data were collected from 11 individuals (4 females, 4 males, and 3 anatomically female goats (intersex) were chosen for PCR analysis using *ZFX* and *SRY* specific primers sequence. For this reason, genomic DNA extraction and PCR amplification were done according to routinely available laboratory protocol. Two *ZFX* and *SRY*-specific primer sets were applied for PCR amplification. Interestingly, our results were re-confirmed, and PCR products were amplified by *ZFX* primer yielding a band of 445 bp in both female and intersex goats. Furthermore, *SRY* expressed 169 bp in both male and intersex goats. In summary, these results suggest that *SRY* candidate gene may provide insight into the factors affecting the intersexuality issue in goat breeds.

KEY WORDS goat, intersexuality, polled intersex syndrome, *SRY*, *ZFX*.

INTRODUCTION

Historically, the polledness in goat production was considered a preference and an important economic feature by farmers. However, the consequence of this on-farm breeding goal was simultaneously an increasing frequency of intersex syndrome (Ramadan and El Hassan, 1988; Ramadan *et al.* 1991). The intersex mechanism under the influence of one or more genes on the Y chromosome induces an undifferentiated gonad to become a testis. It is stressed by the expression of chromosomal aberrations. Some individuals in goat breeds with XX chromosomes develop testicles. In humans, most of them are known as the XX male. This phenomenon is caused by the abnormal exchange of specific X and Y DNA during paternal meiosis (Seboun *et al.* 1986; Petit *et al.* 1987). In contrast, most XX males with genital ambiguities are considered as negative Y (Abbas *et al.* 1990). In addition to, X-dependent and autosomal mutations in the sex determination pathway may explain this phenotype. Abnormal sexual development has been reported in farm animals (Hamerton *et al.* 1969; Eaglesome *et al.* 1979; Hunter *et al.* 1996; Cribiu and Chaffaux, 1990). The intersex phenotype is a complication in which the animal has both male and female sex organs (Ramadan and El Hassan, 1988; Ramadan *et al.* 1991). However, the emergence of PIS (polled intersex syndrome)

in the goat population gradually increased. The natural process of embryonic sex differentiation can be influenced by factors such as the negative harmful maternal environment as well as abnormal growth forces created by mutated genes or the transfer of fetal chromosomes. These destructive disorders may affect the differentiation of the gonads, or external genitalia, and may lead to the birth of an animal that makes it difficult to distinguish sex (male or female). The PIS is a phenomenon in which goats have both male and female sex organs (Ramadan and El Hassan, 1988; Ramadan *et al.* 1991).

Studies of PIS are well documented, several authors have recognized PIS in goats is controlled as a recessive heritable trait that affects only females (XX) genetically and is fully related to the dominant mutation for lack of horns in males and females. Female goats containing PIS are characterized as XX homozygous in terms of karyotype, which are infertile due to different intersex anatomical phenotypes (Pannetier *et al.* 2012). The statistics for the global rate of goat PIS are 4-15%, which poses great challenges for the development of the goat industry due to its impact on reproductive efficiency (Zhan *et al.* 1994; Song *et al.* 2015). The phenotypic identification of PIS cases is challenging. Particularly, because some intersex XX goats without horns cannot be distinguished from normal (XY) males without horns before puberty, therefore a simple molecular test to determine the genetic sex determination is very necessary (Song *et al.* 2015).

Molecular identification methods based on sex-determination genes are suitable tools for the identification of intersex animals (Fábián *et al.* 2017). The Y chromosome is the location of Sex determination genes and may contain 70 to 200 genes (Dhanoa *et al.* 2016). Therefore, chromosome Y plays a fundamental role in male fertility. *ZFY* and *SRY* genes are two important genes in chromosome Y. Candidate gene approach is suitable tool for understanding the genetic mechanism of disorders in mammals (Strah and Kunej, 2019). The *ZFY* gene was originally an autosomal receptor and was transferred to X and The Y chromosome. *ZFX* is the *ZFY* homologue that encodes similar proteins, and it is Self-regulatory of hematopoietic stem cells and embryonic cells (Palmer *et al.* 1990; Galan-Caridad *et al.* 2007). In goats, *ZFY* gene consists of 10 exons (Xiao *et al.* 2021). The *SRY* is testosterone regulatory factor (TDF) regulator, intron-free, and has only one exon in goat. *SRY* is expressed in somatic cells of the genital prominence, while expression continues in sheep (Sekido, 2010; He *et al.* 2019; McElreavey *et al.* 1993; Montgomery *et al.* 1996; Graves, 2015).

Understanding the complexity of this phenomenon at the DNA level may help to discover the molecular mechanisms of sexual differentiation in this species.

To our knowledge, no study has yielded on the identification of PIS goats through a molecular test in Iranian goats. With this motivation, the main goal of the present report was identification of PIS goat anatomically female goats based on *ZFX* and *SRY* sex determination candidate gene findings.

MATERIALS AND METHODS

Animals and sampling

To perform this research, 11 individuals (4 females, 4 males, and 3 anatomically female goats (intersex), overall, were chosen for PCR analysis using ZFX and SRY specific primers sequence. Figure 1 indicated an overview of intersex female goats for molecular investigation in this study.

Molecular analysis

The genomic DNA was purified from blood through conventional protocol (Shams *et al.* 2011). Gel monitoring and NanoDrop 2000/2000c spectrophotometer methods (Thermo Fisher Scientific, USA) were used for quality and quantity measurement tests. Table 1 displays the characteristics of candidate loci, primer sequence, and amplicon size.

PCR amplification was performed in a total volume of 15 µL containing master mix kit (Ampliqon, Denmark) 7.5 µL master mix 2X, 1 pmol of each primer (Forward and Reverse), and 4.9 µL ddH_2O, and 1.5 µL of genomic DNA (all these steps were done on ice). Model of PCR Machin for amplification of fragments was TProfessional Basic Thermocycler by Biometra Ltd.

The PCR products were determined on 1.5% agarose gels stained with metaphor in 1X TBE (Tris, Borate acid, EDTA) buffer. For stating of gel, Ethidium bromide, and for photography UV transilluminator system (Model Tusgene, Iran) was applied.

RESULTS AND DISCUSSION

The phenotypic identification of PIS cases is challenging (Song *et al.* 2015). In particular, before puberty, some female goats without XX bisexual goats cannot be distinguished from normal (XY) males without horns. Therefore, performing a molecular test to determine genetic sex and genotype is very important.

Interestingly, our results were re-confirmed, and PCR product amplified by *ZFX* primer yielded a band of 445 bp in both female goats and the intersex goat. Furthermore, *SRY* expressed 169 bp in both male goats and intersex goat. In summary, these results suggest that *ZFX* and *SRY* candidate genes may provide insight into the factors affecting the sex determination. Figures 2 and 3 illustrated DNA extraction quality and quaintly in 0.8% agarose gel.

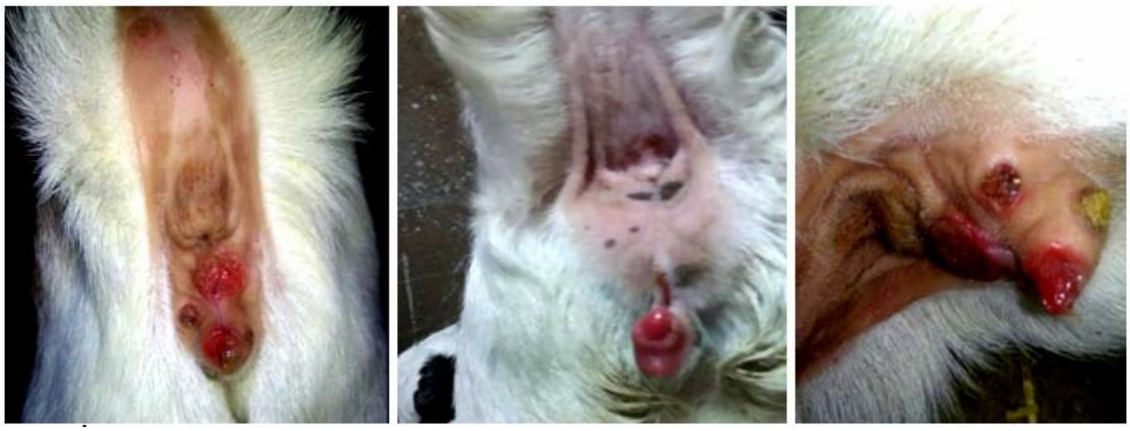

Figure 1 Overview of intersex female goat for molecular investigation in this study

Table 1 Characteristics of candidate loci, primer sequence, PCR product, and amplicon size

Loci	Primer sequence	PCR product	Annealin(g)	References
SRY	5′-CATTGTGTGGTCTCGTGAA-3′ 5′-TGTCTCGGTGTATAGCTAG-3′	169 bp	55	Pailhoux *et al.* (1994)
ZFX	5′-ATAATCACATGGAGAGCCACAAGCT-3′ 5′-GCACTTCTTTGGTATCTGAGAAAGT-3′	445 bp	60	Aasen and Medrano (1991)

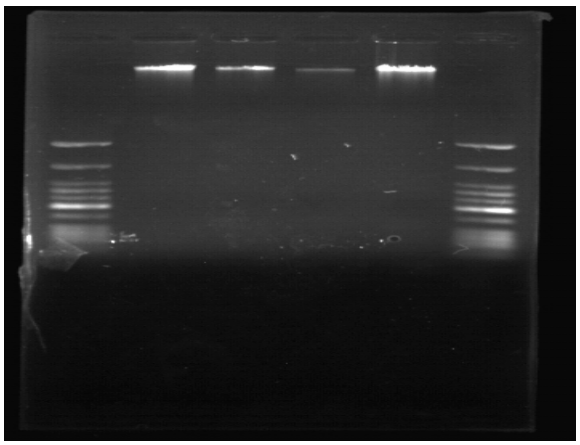

Figure 2 DNA extraction quality and quaintly in 0.8% agarose gel electrophoresis

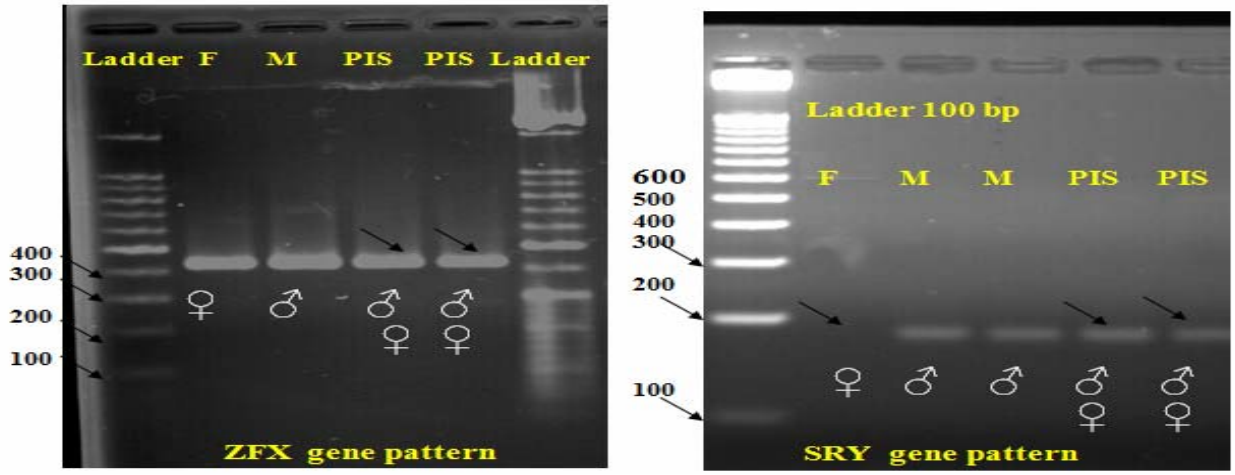

Figure 3 *ZFX* and *SRY* gene electrophoretic pattern between true male and female versus intersex female goat

The prevalence of intersex phenotype in goats varies depending on the breed, population structure and history of origin, and implementation of the breeding method by breeders.

Among domestic goats, false male hermaphrodites are more common in dairy breeds than hairy breeds. Their prevalence is very low among horned wild goats, while it occurs at a high rate in genetically selected breeds for twinning and without horns (Bosu and Basrur, 1984).

Early in the last century, the prevalence of intersex in the Saanen and Toggenburg breeds (bred in the USA) reached 11% and 6%, respectively. Recent studies of crossbred European goats in different parts of India indicate that the prevalence of intersex is below 2% (Zhan *et al*. 1994).

In the same line as Hafez *et al*. (2005) our results reconfirmed, and PCR product amplified by *ZFX* primer yielded a band of 445 bp in both female goats and the intersex goat. Furthermore, *SRY* expressed 169 bp in both male goats and intersex goat. In most mammals, gonad differentiation depends on the presence or absence of the *SRY* gene, the sex-determining region of Y chromosome (Wang and Zhang, 1993). Further investigation is required to establish the correlation between sex-determination genes and intersex traits in goats. This assumption might be addressed in future studies.

The limitations of the present study naturally include a low sample size for intersex animal and also only two sex determination candidate gene was applied for differentiation between normal animals versus intersex female individuals.

CONCLUSION

In goats, the polled intersex syndrome (PIS) kader mutation is responsible for both the absence of horns in males and females and sex-reversal affecting exclusively XX individuals. Understanding the complexity of this phenomenon at the DNA level may help to discover the molecular mechanisms of sexual differentiation. In the present study, PCR product amplified by *ZFX* primer yielded a band of 445 bp in both female goats and the intersex goat. Furthermore, *SRY* expressed 169 bp in both male goats and intersex goat. In summary, these results suggest that *ZFX* and *SRY* candidate genes may provide insight into the factors affecting sexual determination. On this basis, we conclude that *SRY* sequence is suitable tool for the identification intersex female goat and produce 169 bp male-specific bands in DNA test. Further investigation is required to establish the correlation between sex-determination genes and intersex traits in goats.

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