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Follicle stimulating hormone receptor gene polymorphism and its association with fecundity in goats

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Follicle stimulating hormone (FSH) is a biological macromolecule which cannot permeate through cell membrane, so its effect on target cells must be mediated by FSH receptor (Gharib *et al.* 1990). The FSH-FSHR system relays neuronal signal from hypothalamus to the gonads and induces feedback signals to the hypothalamus and pituitary, which keeps the endocrine balance in the reproductive axis and maintains follicle growth, development, differentiation and maturation (Nieschlag *et al.* 1999, George *et al.* 2011). FSH causes proliferation and differentiation in granulose cells. The function of follicle stimulation hormone relate with the FSH receptor (Tisdall *et al.* 1995). The diverse responses of granulosa cells to FSH during follicle growth (Wayne *et al.* 2007) are likely a consequence of the receptor to which FSH binds (Sairam and Babu 2007). FSHR is found on granulosa cells of developing follicles. The binding of FSH to the FSHR stimulates granulosa cell division and steroidogenesis. The polymorphisms in follicle stimulation hormone receptor genes were identified and 3 genotypes observed (AA, AB and BB). Nazifi *et al.* (2015) reported the significant effect of FSHR marker sites on litter size in Baluchi sheep population. The polymorphism of FSHR gene at 3 cleavage sites (304 bp, 214 bp, and 90 bp) within the amplification fragment was observed and 3 genotypes, viz. BB (304 bp) twin birth, AA (214 bp/90 bp) single birth, and AB (304 bp/214 bp/90 bp) twin birth were detected in local Iraqi goat (Hatif *et al.* 2017). To date, there is paucity in research regarding associations of FSHR polymorphisms with fecundity in India. Therefore, this study aimed to elucidate the effects of polymorphisms of FSHR gene on fecundity in goats.

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Pluriparous goats (73) in between second to fifth parity with history of breeding 30 days before were selected. A real time B-mode ultrasonography machine (ALOKA SSD-500) equipped with a linear array 7.5 MHz transrectal scanner was used for reproductive scanning as well as diagnosis of early pregnancy and twinning in goats. Goats were restrained manually in standing position. With gloved hands, the carboxymethylcellulose (CMC) gel was inserted in rectum and faeces were removed completely. The 7.5 MHz linear probe was made rigid by passing hollow plastic pipe and well lubricated probe was inserted through anus into rectum. The tip of the transducer was lubricated with CMC gel. The location of urinary bladder visualisation which was anechoic, taken as landmark point and subsequently probe was moved in forward lateral side in both the directions. Probes was rotated 90 degrees clockwise and counter clockwise to observe the uterine horns entirely. In pregnant goats, the embryonic vesicle along with embryo was detected in single while in twin pregnancy embryonic vesicles along with embryos were detected with trans-rectal approach. In non pregnant goats, the scanned area showed hypo-echoic uterine horns. Relevant images were freezed and prints were taken by using thermal printer. Tran-rectal ultrasonography scanning was completed in each goat within 3 to 5 min. Only 40 pregnant goats out of total 73 goats, were selected.

Blood sample (10 ml) was collected aseptically by jugular vein puncture, into a vacutainer tube containing EDTA as an anticoagulant from 40 pregnant goats. Genomic DNA was extracted from the collected blood samples using DNA extraction Mini kit and the quality and the purity of the DNA were checked and quantification was done by agarose gel electrophoresis. FSHR gene specific primers were custom synthesized and utilized in the study to amplify a segment with 304 bp length of FSHR gene. A pair of PCR primers (forward: 5-CCCATCTTTGGCATCAGC-3 and reverse: 5-ACACAGTGATGAGGGGCAC-3) was used (Hatif *et al.* 2017). PCR was conducted in 25 µL

volume containing approximately 50–100 ng genomic DNA, 12.5 µL Dream taq Green PCR master mix, 0.75 µL each of forward and reverse primers (20 pmole/µL), 8 µL of nuclease-free water. The thermal cycling included initial denaturation at 95ºC for 5 min, followed by 35 amplification cycles of denaturation at 94ºC for 45 sec, annealing at 56ºC for 30 sec, extension at 72ºC for 1 min, with final extension at 72ºC for 10 min. PCR amplification was confirmed by electrophoresis in 2% agarose gel. The DNA fragments were stained with ethidium bromide and the amplified product was visualized as a single compact fluorescent band of expected size under UV light.

Digestion reactions were carried out in final volume of 15 µL containing 7 µL of each PCR products were treated with 0.2 µL of each MSCI restriction enzyme, 1 µL buffer and 6.8 µL nuclease free water. The PCR product for FSHR gene was digested by restriction enzyme MSCI using above protocol and kept in water bath for 12 h at 37°C. Enzyme digestion was analyzed by electrophoresis in 2% agarose gel and the gel was stained with ethidium bromide, and the amplified product was visualized a fluorescent band of expected size under UV light. Statistical analysis was carried out by using ANOVA (Analysis of variance) (Snedecor and Cochran 1989).

Out of 73 goats examined, 40 goats were found pregnant with transrectal ultrasonography on day 30 and 35 post breeding, respectively. All the examined goats by transrectal ultrasonography showed embryonic vesicle, proper embryo and heart beats of embryo on day 30 as well as 35 post breeding, respectively. Out of 40 pregnant goats, 26 goats showed single embryo while 14 goats showed twins on day 30 post breeding while 22 goats showed single embryo and eighteen goats showed twins embryo on day 35 post breeding. In the present study, the detection of twins was 100% accurate on day 35 post breeding which is in concurrence with result of El-Zarkouny *et al.* (2008) and Padilla-Rivas *et al*. (2005). Hence, fetal number may be detected on day 35 with transrectal approach. The transrectal ultrasonography for early pregnancy diagnosis and fetal number is best technique which is easily applicable without risk for goat. The does were examined in standing position with proper restraining by single person only and there was no distress, no rectal bleeding or interruption of pregnancy was observed. The technique is least time consuming as only 3 to 5 min are required for complete examination of 1 doe. Hence, transrectal ultrasonography can be very well adopted at field level and more number of does can be examined with in short period of time. The observation reported in present study are in agreement with Martinez *et al*. (1998), Medan *et al.* (2004), Suguna *et al.* (2008) and Goel and Kharche (2018) as trans-rectal ultrasonography is recommended tool for early pregnancy diagnosis as well as detection of twinning in goats.

In our study, 2 different genotype AA and AB having amplification site (214 bp, 90 bp) and (304 bp, 214 bp, 90 bp) were detected. Our result is not in agreement with Hatif *et al.* (2017) who observed AA, AB and BB genotype in local Iraq goat. In our study, in AA genotype, 21 single and 9 twins were observed while 1 single and 9 twin embryo were observed in AB genotype. The percentage of single and twins was 70% and 30% in AA genotype, and 10% and 90% in AB genotype, respectively (Table 1). Hatif *et al.* (2017) observed 50% and 40% twinning in AA and AB genotype.

Table 1. Distribution of FSHR gene polymorphism and association of AA and AB genotype with litter size in goats

Genotype	Single embryo	Twin embryo	Total litter size	Mean	≠t¹ stat	¹t¹ table
AA (n=30)	21 (70%)	09 (30%)	39	1.30[a]	3.775	2.712
AB (n=10)	01 (10%)	09 (90%)	19	1.90[b]		

Means bearing different superscript within the column differ significantly (P<0.01).

The average litter size was 1.3 and 1.9 in AA and AB genotype, respectively. The result were statistically significant at P<0.01 (Table 1). Guo *et al.* (2013) reported 2.98±0.14, 2.52±0.18 and 1.95±0.21 litter size in CC, CD and DD genotype, and 5' regulatory region at 251 bp of FSHR gene significantly influenced litter size in Jining Grey goat. Zhu *et al*. (2007) reported that litter size in does with genotype BB was significantly higher than does with genotype AA and AB of caprine FSHR gene in Guizhou Black goats. However, the mutation of FSHR had no significant effect on the litter size in either Xiangdong Black or Nanjiang Brown does. Nazifi *et al.* (2015) reported that BB genotype showed higher mean litter size (1.56) than AA genotype in ewes. Also it was reported that association analysis of genotype has shown significant effect on litter size only in Baluchi sheep breed for FSHR gene. In our study, the AB genotype showed statistically significant litter size than AA genotype indicating that FSHR gene plays an important role in regulation of litter size. Xiang *et al.* (2017) reported that genotype BB was significantly higher than AA and AB of QianBei Ma goat and concluded that FSHR gene might play an important role in regulation of litter size and provide basic data for exploring candidate gene effecting reproductive traits. Wang *et al.* (2015) reported FSHR polymorphisms could be used as genetic markers in multi-gene pyramiding for improving litter size in sheep.

SUMMARY

Pluriparous goats (73) in between second to fifth parity with history of breeding 30 days before were selected for present study. A real time B-mode ultrasonography machine equipped with a linear array 7.5 MHz transrectal scanner was used for diagnosis of early pregnancy and twinning in goats. Out of 73 goats, 40 were detected pregnant with 22 goats with single embryo and 18 goats with twin embryos. Genomic DNA was extracted from the blood of all pregnant goats and polymerase chain reaction (PCR) with FSHR gene

specific primers was carried out. The PCR product for FSHR gene was digested by restriction enzyme MSCI. Two genotypes AA and AB having amplification size (214 bp, 90 bp) and (214 bp, 90 bp and 304 bp) were detected, respectively. In AA genotype, 21 single and 9 twins were observed while 1 single and 9 twin embryos were observed in AB genotype. The percentage of single and twins was 70% and 30% in AA genotype, and 10% and 90% in AB genotype. In AA genotype, the average litter size was 1.3 while in AB genotype it was 1.9 and the difference was statistially significant. The AB genotype for the FSHR gene is responsible for more twining percentage.

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