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Ovarian Expression Profile of KISS1 and NGF Genes in Indian Goat

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ABSTRACT

Background: Litter size is one of the most important economic traits that translates into higher returns for farmers. KISS 1 and NGF genes plays significant role in female reproduction. The purpose of the study was to evaluate the potential variation in the of expression levels of KISS1 and NGF genes in ovarian tissue of two different goat breeds with diverse prolificacy.

Methods: Phenotypic records pertaining to litter size of Barbari and Jamunapari goats were collected. Total RNA was extracted from both breeds' ovarian tissues for real-time PCR quantification of the KISS1 and NGF genes, using GAPDH and β-actin as endogenous controls. The target gene's expression levels were measured and the fold-change was determined.

Result: The phenotypic recording revealed that Barbari goats are more prolific in nature than Jamunapari goats. The expression levels of KISS1 and NGF genes were higher in Barbari goat and lower in Jamunapari goat ($P<0.05$). The results of this study can be used to further discover the crucial role of KISS1 and NGF genes in reproduction for improving the prolificacy in goats.

Key words: Barbari, Gene expression, Jamunapari, KISS1, NGF, Prolificacy.

Increasing the litter size is of great importance to goat keepers and thus in goat breeding prolificacy is one of the economically important traits. In reproduction, KISS1 and the NGF genes play a key role. KISS1 gene is responsible for multiple type of birth and could be considered as a candidate for selection of reproductive traits (Messager *et al*. 2005; Richard *et al*. 2008; Gaytan *et al*. 2009; An *et al*. 2013; Clarke *et al*. 2015). KISS1 has also shown to regulate seasonal reproductive activity in Syrian hamsters and sheep (Revel *et al*. 2006; Revel *et al*. 2007; Clarke *et al*. 2009). Significant increase ($P<0.05$) of KISS1 mRNAs was associated with puberty goats (Samir *et al*. 2018). Similarly, NGF gene and its relationship with reproductive traits such as superior ovulation rate and litter size in farm animals have been studied to aid in the selection of multiparous animals (Menchaca *et al*. 2002; Akpa *et al*. 2011, Naicy *et al*. 2016). NGF plays a critical role in development of the mammalian ovary, oogenesis and follicular synthesis (Lavasani *et al*. 2006; Julio-Pieper *et al*. 2009). Previous researcher has associated higher expression levels of ovarian NGF mRNA with prolificacy in goats (Naicy *et al*. 2016). The interaction between NGF and gonadotrophins in bovine oviduct was reported where NGF could increase the expression of FSHR and LHR in oviduct epithelial cells *via* its effects on its receptor *i.e*. NTRK1 (Li *et al*. 2014). Although the importance of KISS1 and NGF for reproduction is apparent, these genes have not been properly evaluated as a candidate for genetic reproduction in animals, especially in prolific small ruminants. Therefore, the current study was aimed to determine the relative expression of mRNA expression of KISS1 and NGF genes in the ovaries of two Indian goat breeds with differing litter size.

The present investigation was conducted in two different goat breeds i.e. Barbari and Jamunapari maintained at the ICAR-Central Institute for Research on Goats (CIRG) Makhdoom. Barbari goat (1.51) is relatively more prolific than the Jamunapari goat (1.34) (Chauhan, 2016). The female goats with age group of 2-5 years were selected for sample collection. Ovary tissues were collected, washed twice in phosphate buffer saline and stored in RNAlater (Sigma) at -70°C for future use. The total RNA was isolated using Trizol method and quality was checked by UV VIS photometer (Eppendorf). The first strand cDNA was synthesized using Transcriptor first strand cDNA synthesis kit (TAKARA). Real Time PCR analysis was carried out in Light Cycler 480 (Roche Applied Science, Indianapolis, IL, USA) using SYBR Green® master mix (TAKARA) as per manufacturer instructions. A set of primers for KISS1 gene (F 5'-TGAACGTGCTGCTTTCCT-3' and R 5' GCCTGTG GTTCTAGGATTCTC-3') and for NGF gene (F-5' GGGAGA

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GGTGAACATCAACAA-3’andR-5’-GTCGTGGTACAATAC GAGTTCC-3’) were used. Two endogenous reference genes namely GAPDH (F-5’-GCAAGTTCCACGGCACAGTC’-3 and R-5’ -CCC ACTTGATGTTGGCAGGA-3’)andβ-actin(F-5’ TGCCCTGAGGCTCTCTTCCA-3’ and R 5’ -TGCGGATG TCGACGTCACA-3’) were used to normalize the gene expression of KISS1 and NGF genes. The thermal profile was standardised as initial denaturation at 94°C for 10 min, followed by 45 cycles, denaturation at 94°C for 10 sec, annealing at 62°C for 15 sec, and extension at 72°C for 20 sec. All the samples were taken in duplicate and a non-template control was run simultaneously. Fold-change in expression level of the target genes were presented using E-method (Livak and Schmittgen, 2001). The crossing point (Cp) readings for each unknown sample were then used to calculate the amount of either the target or housekeeping gene using the second derivative maximum method with the Light cycler 480 analysis software version 1.5 (Roche Applied Science, Indianapolis, IL, USA). The geometric mean of the resulting ratios was calculated,

e.g. $T1/R(all)=$

$$(T1/R1\times T1/R2)^{1/2} \text{ and } T2/R(all) = (T2/R1\times T2/R2)^{½}$$

Where, T- Target gene, R- Reference gene

The relative ovarian tissue abundance of KISS1 and NGF mRNA among two breeds were determined using quantitative real time PCR. The relative expression of KISS1 and NGF genes was higher in ovaries of Barbari than Jamunapari goats (Fig. 1).

The expression pattern of the KISS1 gene revealed that Barbari and Jamunapari had 2.275 and 0.007 fold gene expressions, respectively, than the control. Similarly, the expression pattern of the NGF gene indicated that the Barbari breed had higher expression (3.237 fold) than the control while the Jamunapari breed had 1.749 fold expression .The cross point (CP) value showed substantial effect ($P<0.05$) among the breeds. The expression levels of KISS1 and NGF genes between the Barbari and Jamunapari goats were markedly different. Many researchers have shown the association of KISS1 gene polymorphism with litter traits in goats (An *et al*. 2013; Sahoo *et al*. 2019). Various earlier studies identified that Kisspeptin regulate GnRH and gonadotropin secretion and the gene expression of KISS1 has important role in animal reproduction (Navarro *et al*. 2005; Silveira *et al*. 2010; Navarro *et al*. 2012; Choe *et al*. 2013; Vargas *et al*. 2017). Our finding of higher ovarian expression of NGF gene was in agreement with earlier report where NGF expression in ovaries of goats with history of multiple births had higher than singles in goats (Naincy *et al*., 2016). The finding of the current investigation implies the importance of KISS1 and NGF genes in goat reproduction and consequently prolificacy.

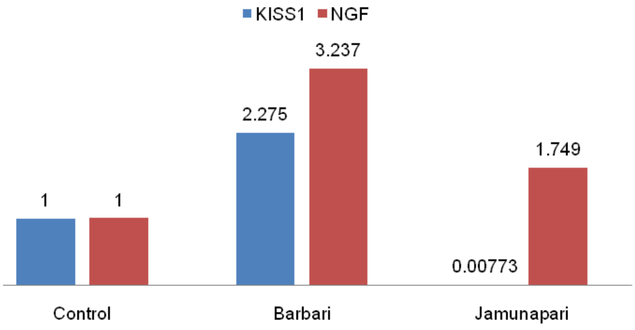

Fig 1: Transcript relative abundance of KISS1 and NGF gene in Barbari and Jamunapari goat.

CONCLUSION

The Barbari goat showed relatively higher levels of ovarian mRNA expression for the KISS1 and NGF genes in comparison to the Jamunapari goat. The increased expression of the KISS1 and NGF genes in more prolific goats could be linked to larger litter sizes. Overall, the KISS1 and NGF genes are associated to female reproduction and could be candidates for litter size trait. However, because these are preliminary findings, more research in a larger population and with diverse breeds is required to validate them.

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