EVALUATION OF UTERINE EXPRESSION OF *SIRTUIN3 (SIRT3)* mRNA IN MALABARI AND ATTAPPADY BLACK GOATS OF KERALA*

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Abstract

The sirtuins are a family of highly conserved NAD+-dependent deacetylases which are potent cellular regulators and have been implicated as key inhibitors of inflammation. Sirtuin3 (SIRT3),a member of the sirtuin family, prevents the activation of reactive oxygen species (ROS) thereby protecting the cell from oxidative stress. A study was conducted to compare the relative abundance of SIRT3 mRNA in uterine tissues of two native goat breeds of Kerala, Malabari and Attappady Black. The mRNA isolated from uterine tissues of Malabari and Attappady Black goats weresubjected to quantitative PCR (qPCR). The relative abundance of SIRT3 mRNA was significantly ($p<0.05$) higher in Attappady Black goats when compared with Malabari goats.SIRT3 has a protective role on the uterus by preventing the activation of reactive oxygen species (ROS) and thus preventing inflammatory process. The results of the present study suggest that SIRT3 gene plays an important role in the protection of uterine tissues in Attappady Black goats by its upregulation in the uterine tissues and can be considered as a potent candidate gene for reproductive traits in goats.

Keywords:*Sirtuin3; Malabari goats, Attappady Black, uterine expression.*

The mammalian sirtuins are a family of NAD-dependent enzymes with homology to the *Saccharomyces cerevisiae* gene silent information regulator 2 (*Sir2*). The sirtuin family contains seven members named *SIRT1-7* having different cellular localization and target

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proteins (Nogueiras*et al*.,2012). SIRT3 has emerged as a mitochondrial fidelity protein that directs energy generation and regulates ROS scavenging proteins (Zhao *et al*.,2016). It is central to the control of metabolic processes and also has a critical role in fertility by preventing the activation of reactive oxygen species (ROS). Moreover SIRT3 can regulate the acetylation of several proteins, often at multiple sites across several metabolic pathways, which include fatty acid oxidation, ketogenesis, amino acid catabolism and tricarboxylic acid cycles, as well as mitochondrial regulatory proteins (Rardin*et al*., 2013) and can directly modulate oxidative stress through deacetylation and activation of superoxide dismutase (Tao *et al*., 2013).

Goat production in Kerala is mainly centered on its native breeds. The two important native breeds of goats in Kerala are Malabari and Attappady Black. Malabari goats are well known for their prolificacy, growth rate and milk production. Attappady Black goats which are mainly reared by the tribal community of Attappady region of Palakkad district are known for their sturdy nature and growth rate (Kaura, 1952 and Stephen *et al*.,2005). Since uterine environment play a critical role in implantation, pre natal growth and early kid mortality, aresearch was conducted with an objective to study the relative uterine expression of *SIRT3*, which has a critical role in fertility by preventing the activation of reactive oxygen species (ROS).

Materials and Methods

In this study, tissue samples of uterus were obtained from Malabari(n=6) and Attappady Black (n=6) goatsof 4 to 5 years of age from the Kerala Veterinary and Animal Science University Meat Plant and were stored in RNA later (Sigma-Aldrich) at -80˚C till RNA isolation.

Total RNA extraction

Total RNA was isolated using TRI reagent(Sigma Aldrich) and treated with DNase I (Sigma Aldrich) to prevent DNA contamination. The concentrations were measured by using spectrophotometer(NanoDrop™ 2000C) and integrity was verified using 1%agarose gel electrophoresis.

Complementary DNA (cDNA) synthesis

The cDNA was synthesized using RevertAid First strand cDNA synthesis kit (Thermo scientific). 500 ng of total RNA was taken for cDNA synthesis in 20 µl reaction volume. The cDNA synthesized was stored at -80 ˚C until further use.

Quantitative PCR (qPCR)

For the present study g*lyceraldehyde 3 phosphate dehydrogenase (GAPDH)* and *beta actin* (*β-actin*)were selected as internal control gene. Using sequences available in NCBI database, primers were designed for amplification of 103 bp region of *SIRT3* (NM_001206669.1; STRT F: 5'TCCCTGACTCAAAGCTCGTT 3'; STRT R: 5'ATCACGTCGGCCCAGAAG 3')from cDNA. Reference gene primers i.e., *GAPDH* and *β-actin* genes were selected as per Naicy*et al*. (2016). Quantitative PCR (qPCR) was performed in triplicate for each sample in Illumina Eco® Q- RT PCR system using SYBR green chemistry. In addition, a non-template control (NTC) for each gene to check for primer-dimers and a negative control (nuclease free water) were also included.

The qPCR reaction was carried out in 12.5 µl mixture containing 6.25 µl of SYBR Green, 10 p*M* each of forward and reverse primer and 25 ng of cDNA as template. The thermal cycling profile for the reaction includes initial denaturation for 3 min at 95˚C, followed by 40 cycles of denaturation at 94˚C for 30 sec, annealing at 54.5˚C, 60˚C and 60˚C for 15 sec for *SIRT3,GAPDH* and *β-actin*,respectively, followed by extension at 72˚C for 30 sec. Dissociation (melt) curve analysis was done after each PCR. The protocol for melt curve analysis was 95^0C for 15 sec, 55^0 C for 15 sec followed by 95° C for 15 sec.

The $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001)was used for calculating relative expression of*SIRT3* gene expression by using *GAPDH* and *β-actin* as internal control.

Results and Discussion

RNA isolated had good integrity and quality. Using conventional gradient PCR, amplification of the target and the reference genes were standardised (Fig. 1). Using Illumina Eco® Q- RT PCR, quantitative PCR (qPCR) was performed and melt curves were generated. Melt curve revealed a single peak for each gene which indicated the absence of nonspecific products. *SIRT3*uterine expression ofMalabaribreed was taken as control. A significantly (P<0.05) higher uterine expression of *SIRT3* was noticed in Attappady Black goats (around four fold) when compared to that in Malabari goats (Fig. 2).

This is the first study on*SIRT3*gene in goats. SIRT3, which is known to be a global regulator of mitochondrial protein acetylation, is capable of coordinating cellular responses to nutrient status and energy homeostasis. Moreover SIRT3 can regulate the acetylation of several proteins and can directly modulate oxidative stress through deacetylation and activation of superoxide dismutase (Tao *et al*., 2013). Inactivation of SIRT3 has been shown to trigger intracellularROS production, mainly of mitochondrial origin (Kawamura *et al.* 2010). Apart from protecting the cell from ROS, SIRT3 is also reported to shield the mitochondria by deacetylation and subsequent inactivation of cyclophilin D protein thereby preventing

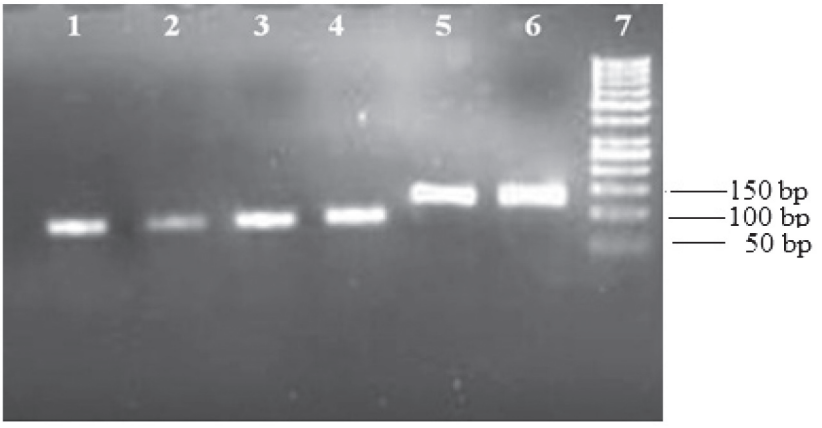

Fig. 1: Agarose gel electrophoresis demonstrating the qPCR products
Lane 1-2: *SIRT3* (103 bp),Lane 3-4: *β-actin*(105 bp),Lane 5-6: *GAPDH* (127 bp),Lane 7: 50 bp DNA ladder

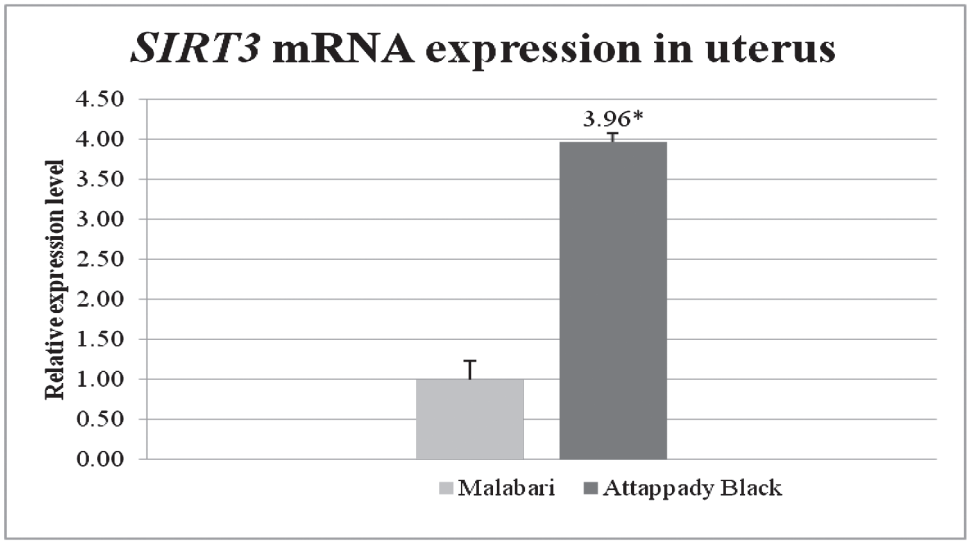

Fig. 2: *SIRT3* mRNA expression level in uterus of Attappady Black and Malabari goat (*p-value≤0.05)

formation of mitochondrial permeability transition pore (mPTP) and hence sustaining mitochondrial membrane potential (Sun *et al*., 2017). These reports suggested the tissue protective role of *SIRT3*. Lim *et al*. (2016) stated that *SIRT3* impedes the production of pro-inflammatory and pro-labour mediators which are induced by known mediators of preterm birth, thereby preventing preterm births.

Uterine environment of the mother play an important role in implantation to early kid survival. Oxidative stress (OS), characterised by an imbalance between pro-oxidant molecules (like reactive oxygen and nitrogen species) and antioxidant defenses can lead to endometritis and spontaneous abortion (Agarwal *et al.,*2012). Hence *SIRT3* gene (SIRT3-a mitochondrial targeting protein), which has a critical role in protecting cells against OS by reducing ROS levels was selected as the candidate gene for the current research to determine its relative expression in two native goat breeds of Kerala. Having originated from the hilly terrains of Attappady region in Palakkad district of Kerala, Attappady Black goats have a reputation of hardy nature and are comparatively thriftier than other goats. They are comparatively more resistantto diseases (Thomas *et al*., 2011).

The results of the present study suggest that *SIRT3* gene is upregulated in the uterine tissues of Attappady Black goats and it plays an important role inprotection of uterine tissues.It could be considered as a potent candidate gene for fertility traits in goats. Further studies are required to explore the association of this gene with reproductive and fertility traits in goats to ascertain the role of this gene in goat reproduction.

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