Short Paper

Estrogen receptor gene 1 expression in caprine and its effect on fertility

Saraswat, S.[1*]; Rout, P. K.[2]; Kharche, S. D.[1]; Goel, A. K.[1]; Jindal, S. K.[1] and Kumar, S.[1]

[1]Physiology Reproduction and Shelter Management (PRSM) Division, Central Institute for Research on Goats (CIRG), Makhdoom, Farah-281122, Mathura, Uttar Pradesh, India; [2]GGB Division, Central Institute for Research on goats (CIRG), Makhdoom, Farah-281122, Mathura, Uttar Pradesh, India

[*]***Correspondence:*** *S. Saraswat, Physiology Reproduction and Shelter Management (PRSM) Division, Central Institute for Research on goats (CIRG), Makhdoom, Farah-281122, Mathura, Uttar Pradesh, India. E-mail: sonia.saraswat@gmail.com*

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Summary

The present study was undertaken to analyze the expression pattern of estrogen receptor 1 gene (ESR1) in Barbari bucks (fertile and non-fertile) identified on the basis of seminal quality traits and fertility trials. RNA was extracted from the spleen by Trizol method. The expression pattern of ESR1 gene was analyzed using real time polymerase chain reaction (RT-PCR). The expression pattern of ESR1 gene was analyzed by RT-PCR (Roche LC-480). Relative quantification by RT-PCR indicated that the ESR1 gene expression showed more fold in fertile bucks as compared to non-fertile.

Key words: ESR1 gene, Estrogen receptors, RNA, RT-PCR

Introduction

Estrogen is a steroid hormone primarily synthesized in ovary and testis (Katsu *et al*., 2010) which regulates a variety of functions in vertebrates, including re-productive, immune and central nervous systems (Vasudevan and Pfaff, 2008; Bakker and Brock, 2010; McCarthy, 2010). At present, few researches (one-two) are available on the biological roles of estrogen in vertebrates (Hewitt and Korach, 2003; Irsik *et al*., 2013). Estrogen is important in the regulation of the male reproductive tract; its importance has been found in male (Irsik *et al*., 2013). Additionally, estrogen works through both genomic (transcriptional regulation through estrogen response elements) and non-genomic pathways (activation of cell-signaling cascades). To date, two estrogen receptors (ERα and ERβ) encoded by different genes have been described (Flouriot *et al*., 2000). The role of ESR1 in buck is poorly understood in Barbari goat breed in India as well as around the world. This work might be helpful to reveal the importance of ESR1 in bucks. The expression of ESR1 gene will support the candidacy of this gene to be used in selection of reproducing goat breeds.

Materials and Methods

All procedures contributing to this work comply with the ethical standards of the institutional guides on the care and use of laboratory animals. Fertile and non-fertile (bucks (n=6)) were taken on the basis of routine semen collection (Saraswat *et al*., 2012). Bucks which were under animal culling order were sacrificed and organs were harvested for gene expression. Expression of ESR1 gene was conducted by use of commercially available kits such as Transcriptor first strand cDNA synthesis (Kit Roche, USA), ROCHE, SYBRR Green qPCRkit (Roche, USA). Amplification gene involved specific primers which were designed from the published sequence (accession No. XM_005684972.1) by the software (Integrated DNA Technologies).

RNA was extracted using Tri-reagent (Sigma) from tissues of fertile (G1) and non-fertile (G2) bucks of Barbari breed. 100 mg tissue was crushed in 1 ml Trizol using homogenizer, tissue extract was taken in 1.5 ml centrifuge tube then chloroform (200 µL) was added and incubated at room temperature for 15 min then centrifuged at 12000 rpm for 20 min at 4°C, upper aqueous layer was transferred into a fresh centrifuge tube, then equal volume of isoproponal was added. After 20 min incubation, again centrifugation was done at 12000 rpm for 20 min at 4°C in order to obtain the RNA pellet. Frequent washing of RNA pellet was done in 70% ethanol at 12000 rpm for 5 min at 4°C. The RNA pellet was dissolved in 20 µL DEPC treated water and incubated at 65°C for 15 min for the complete dissolution of the pellet. The amount and purity of total RNA was checked using the bio-photometer plus (Eppendorf). The quality and purity of total RNA was checked by agarose gel (1%) electrophoresis by visualization under UV light. Furthermore, the first strand cDNA was synthesized from the isolated total RNA using Transcriptor first strand cDNA synthesis kit (Roche, USA) as per manufacturer's instructions. Real

time polymerase chain reaction (RT-PCR) was carried out using reverse transcription system (LC-480, Roche, USA). The quantitative RT-PCR was performed using Roche Applied Science SYBR Master Mix following manufactures instructions. The annealing temperature was standardized using cDNA prepared from mRNA by PCR. The reaction was carried out at different annealing temperatures, primer concentrations, $MgCl_2$ concentration, template DNA and Taq polymerase. The optimum temperature was used in subsequent PCR. Gene expression results were analyzed as the relative abundance level after normalizing with mRNA expression level of housekeeping gene (the GAPDH gene used as a house keeping gene with the following primers-Forward-5′-GTG ATG CTG GTG CTG AGT AC-3′; Reverse-5′-GTA GAA GAG TGA GTG TCG C-3′). The mRNA expression differences were analyzed as per Roche Light Cycler-480 by E method and manual pairing.

Results

The expression of ESR1 gene was higher in Barbari fertile bucks (Table 1). The semi-quantitative reverse transcription PCR result of ESR1 and GAPDH showed remarkable differences among tissues. In a relative quantification plot of ESR1 mRNA expression (Fig. 1), light gray block indicates control (non-fertile buck) and dark gray block indicates BAR-ES (fertile bucks).

Discussion

The physiological role of estrogen in male reproduction was extensively revisited by Carreau *et al*. (2002) but expression study is rare. Through this study for the first time we determine the ESR1 mRNA expression and analysis in Indian goat breed (Barbari). Using semi-quantitative PCR it was found that fold value is higher in Barbari fertile (2.28) buck than that of low fertile bucks (1.00) (control). Lack of fertility is mainly due to the disruption of fluid reabsorption in efferent ductules which increased the backpressure of the accumulating luminal fluids, which leads to a progressive degeneration of the testicular tissue. Fu *et al*. (2014) showed that estrogen receptor gene was expressed in a variety of tissues, which is similar to the ERs in many species. A functional role for estrogens acting via ERa in modulating the contractile activity of the epididymis smooth muscle cells has long been described (Filippi *et al*., 2005; Fibbi *et al*., 2009). However, a greater expression of ERa in the muscle layer of the male genital tract of *A. lituratus* was detected in the regressive period when the luminal sperm were absent. These data suggest that besides contractibility, ERa may be involved in other epididy malfunctions. Indeed a recent study revealed that ERa is essential for proliferation of smooth muscle cells in the rat prostate (Zhou *et al*., 2011) additionally, there is a growing body of evidence that factors arising from the interstitium may be important in stimulating epithelial cells by a paracrine action in diverse organs (Zhou *et al*., 2011). In the liver, the high level of ER transcript and protein levels confirm the primary role of estrogen/ER endocrine system in the hepatic physiological processes, even in trout males. The rtERα1 protein was present in different cells involved in immunity: the endothelial cells of the sinusoids, the Kupffer cells and the epithelial cells of bile duct. These first two cellular types belong to the reticulo endothelial system (RES). The cells which compose this system take up and degrade pathogens (Massart *et al*., 2014). Moreover, the rtERα1 present in the epithelial cells of bile duct are probably not implied in the vitellogenin

Table 1: Pairing and fold value of Barbari spleen and control

Pairing	Targets	Reference	Mean Cp	Cp error	Mean Cp	Cp error	Normalized
Control	ESR	GAPDH	30.94416	6.205362	37.61202	0.170517	1
Barbari spleen	ESR	GAPDH	34.9812	0.861205	22.90378	0.265188	$2.28E^{-06}$

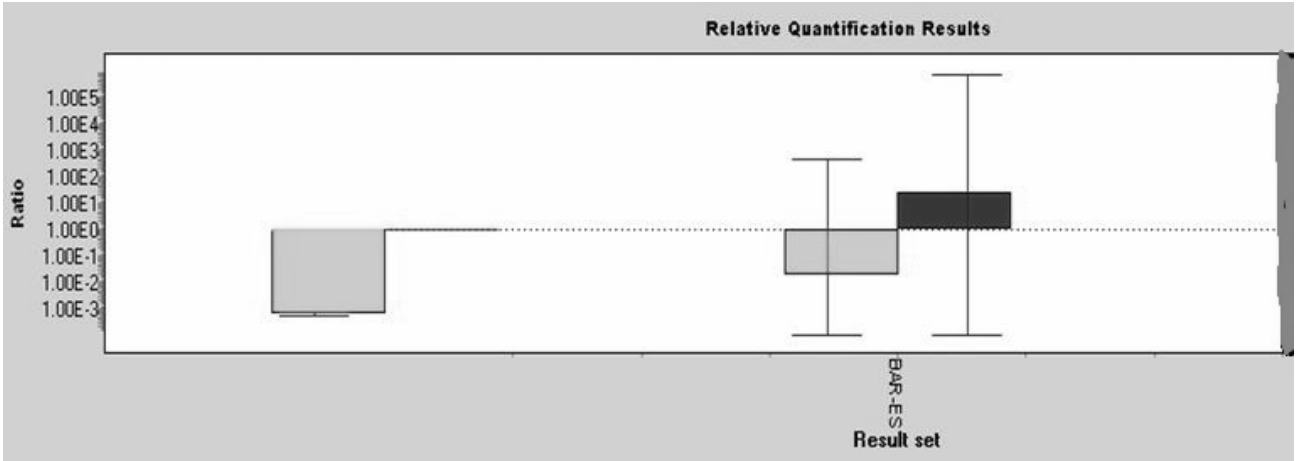

Fig. 1: Relative plot (light gray block indicating control (non-fertile buck) and dark gray block indicating Barbari (fertile bucks)) spleen tissue

synthesis when produced in males in the presence of xenoestrogens, as this protein is not located in the bile duct (Massart *et al*., 2014). In mammals, the transcytosis process is mediated by the polymeric Ig receptor (pIgR), whose expression is regulated by steroids, including estrogens and polypeptide hormones (Beagley and Gockel, 2003; Kaetzel, 2005). The result of this study supports the reproductive functions of ERs in male goat, more fold value was observed in fertile bucksas compared to non-fertile (control).

Estrogen or its receptor gene (ESR1) is an absolute necessity for different functions and in fertility in bucks; this information should fuel future investigation to define the role of ESR1 gene as a candidate gene for better fertility and normal physiology.

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Conflict of interest

We declare no conflicts of interest.

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