International Journal of Animal Science, Husbandry and Livestock Production ISSN: 2141-5191 Vol. 3 (12), pp. 237-241, December 2017. Available online at www.advancedscholarsjournals.org © Advanced Scholars Journals

Full Length Research Paper

Gene expression in mature and immature oocytes and embryos of goats

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Accepted 15 November, 2017

CD44 family belongs to a large group of proteins that bind to hyaluronic acid. It has important role in oocyte maturation, fertilization and embryo development. We analyzed CD44 in oocytes and embryos of goat and different breeds of sheep. We used both Kermani and baluchi breeds of sheep, two Rayeni and Tali goats. Domestic animals kept after feeding for 3 months exude embryos and oocyte. After this, the animals were cured by keeping full progesterone for 14 days in the vagina. Gonadotropin-releasing hormone (GnRH) hormone was injected from the animals' vagina and made ready for sampling after 1 day. After that, mature oocyte was exuded from the animals' uterus and they were taken for slaughter. To get the embryos, male animals were kept beside them. After 6 days these animals were taken for slaughter. In another way, ovary collected from the slaughter house exuded immature oocyte. In the laboratory, immature and mature oocyte was produced. So by keeping sperm under environmental condition embryo culture is produced. The samples exude RNA using kit; after that, cDNA was produced by special protocol. The products produced by conventional PCR and Real time PCR were studied. Result shows that gene expression does not exist in immature oocyte of sheep. In Tali goat, expression of this gene was more than in Rayeni goat.

Key words: CD44 gene, gene expression, real time PCR, goat, sheep.

INTRODUCTION

Glycosaminoglycans (GAGs) play a main role in the proliferation and differentiation of a variety of cell types (Luz et al., 2012). The communication between the granulosa cells and the adjoining is vital for the attainment of oocyte aptitude (Assidi et al., 2008). The growth of cumulus cells might be absolutely connected to the ovulation, fertilization, and subsequent zygote growth (Chen et al., 1993).

Among the GAGs, hyaluronic acid (HA) is a lofty molecular weight polysaccharide found in the extracellular matrix of most animal tissues and is one of the most

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Table 1. Primer sequence to Real time PCR used to notice the presence of CD44 receptor in sheep and goat immature and mature oocytes and embryos.

Gene	Nucleotide sequence
CD44	5-CAACACCTCCCASTATGACAC-3
	5-TTCTTCTGCCCACACCTTCT-3
b-actin	5-CAACTGGGACGACATGGA-3
	5-TGGTGGTGAAGCTGTAGC-3

abundant GAGs in the uterine, oviductal andfollicular fluids (Archibong et al., 1987). Through the progression of ovulation, cumulus cells exude HA (Salustri et al., 1990). Moreover, HA adds to the average development of 1 and 2-cell porcine embryos (Miyano at al., 1994) as well as *in vitro* bovine embryo growth at the blastocyst stage (Furnus et al., 1998). HA mostly binds to CD44, which is a glycol-protein extensively expressed on the outside of many mammalian cells. CD44 exists as manifold isoforms expressed in an exact way for different cell types. These isoforms affect splicing and post-translational modifications, where they can be glycosylated in a different way (Opela et al., 2012). Cell exterior glycoprotein CD44 is present in mature oocytes and embryos in small number of classes of mammals such as bovine (Furnus et al., 2003). CD44 was not detected in immature oocytes in porcine (Yokoo et al., 2007). This information shows that CD44 is expressed throughout the maturation procedure, which suggests its significance in this phase. It is reasonable to assume that HA profile is directly proportional to the amount of CD44 in somatic cells surrounding the rising oocyte (Luz et al., 2012). There is a whole connection loss of cumulus growth in cumulus-oocyte complexes (COCs) and oocyte meiotic series (Allworth and Albertini, 1993). The growth of cumulus cells might be absolutely connected to the ovulation, fertilization, and subsequent zygote growth (opelia et al., 2012).

The purpose of this study was to investigate whether this gene is expressed in immature oocytes and embryos of goats.

MATERIALS AND METHODS

Natural ways for collecting mature oocytes and embryo

This procedure was performed as follows: First, estrous cycle synchronized was created in the vagina with a sponge containing 60 mg medroxyprogesterone acetate for 14 days. After 24 h, sponge was used to remove estrus from rams. After 90 h when the sheep was slaughtered, the estrus extracted was placed in the vagina. A syringe was then placed in a liquid at the lab; the fetus was confirmed under a microscope with a magnification of 10 to 50X; for only the morula stage embryos. In goats, estrus synchronization was done using CIDR and injections for 14 h; CIDR removal was performed in 5/2 ml of GnRH for 48-24 h; after CIDR was used to remove heat from the goats. For every 10 female goats, a male goat was used for mating them just like the other procedures used for sheep.

In vivo embryo production

Five hair ewes and goat (2 to 6 years old) were submitted for similar hormonal action as described above. Females were mated at the start of estrus and 24 h later, rams and male goat were used to form fertility. Recovery of embryo was performed by laparotomy for six and seven days during the first mating. Soon after genital area contact, every uterine horn was washed with 25-30 ml DMPBS. Embryo excellence and growth phase were evaluated under a microscope at 10 to 50X magnification. Embryos at the morula phase were then frozen (-80°C) using Real time PCR.

RNA

RNA was extracted using RNA Purification Kit, after which CDNA synthesis was carried out. In this protocol, the materials and reactions added to a system of numbered and color-coded labels are shown. Replication done using PARSGENOME MiR-Amp kit includes a three-step protocol.

Real-time PCR method

Replication done using PARSGENOME MiR-Amp kit includes a three-step protocol; the cDNA amplification was conducted with Real time using PCR primers to increase the specificity and yield of the PCR product (Table 1). Sum of RNA was remote as described above (Opiela et al., 2012). The comparative expressions levels of glyceraldehyde-3-phosphate dehydrogenize were used for normal marker gene expression in all samples. SYBR Green PCR Master Mix Kit (PARSGENOME, Iran) was used to do relative quantification of gene expression. Every reaction (total volume of 20 µL) consisted of total RNA (2 ng/µL), 1× of SYBR Green PCR master mix containing an optimized RT-PCR buffer, 2.5 nM of MgCl2, nucleotides, Taq DNA polymerase, SYBR Green and stabilisers, 200 nM each of the forward and reverse primer (Luz et al.2012), and 1× of RT/RNase block enzyme mixture. Thermal cycling conditions are as follows: 35 min at 50°C (for the first-strand synthesis); 12 min at 95°C; 40 cycles of 30 s at 95°C for denaturing; 60 s at 60°C for annealing; and 30s at 72°C for extension. Experiments were carried out using Master cycler apparatus (Eppendorf, UK Limited, Cambridge). GAPDH was used as an endogenous normal. The results for individual target genes were consistent with the relative endogenous standard. Each reaction PCR was sprinted in triplicate and the obtained results were averaged. Ct method was used for calculating the comparative quantification. Statistical analysis differences in transcripts level were assessed using ANOVA test.

RESULTS AND DISCUSSION

Quality and quantity of DNA and RNA were extracted from high contamination and bands were observed on agarose gel, using spectrophotometer. The Quantity and quality of extracted DNA were calculated and recorded (Figure 1). In the original Eppendorf tube, mix temperature gradient was attempted using Real time PCR. The mature oocytes of Kermani and Baluchi sheep were lower than that of Rhine goats; CD44 genes wereencoded at a temperature range of 55 to 60°C. CT criteria for selecting optimal binding temperature were high, and few Rn at 59.5 and 60.7°C for CD44 gene was selected.

The graphs show Ct at different temperatures and ct is the cycle that begins the Sigma growth chart. The melting

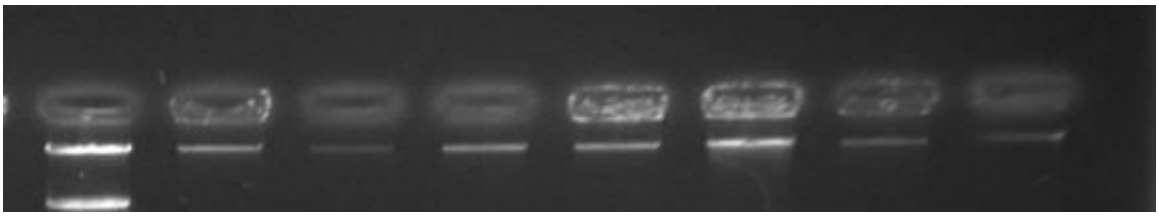

Figure 1. RNA was extracted from the agarose gel shows

Table 2. Results of thermal gradients CT internal control and CD44 gene.

Temperature gradient	Internal control gene CT	CD44 gene CT
75	23.2	1.92
7.91	22.6	1.981
829.	21.8	13942

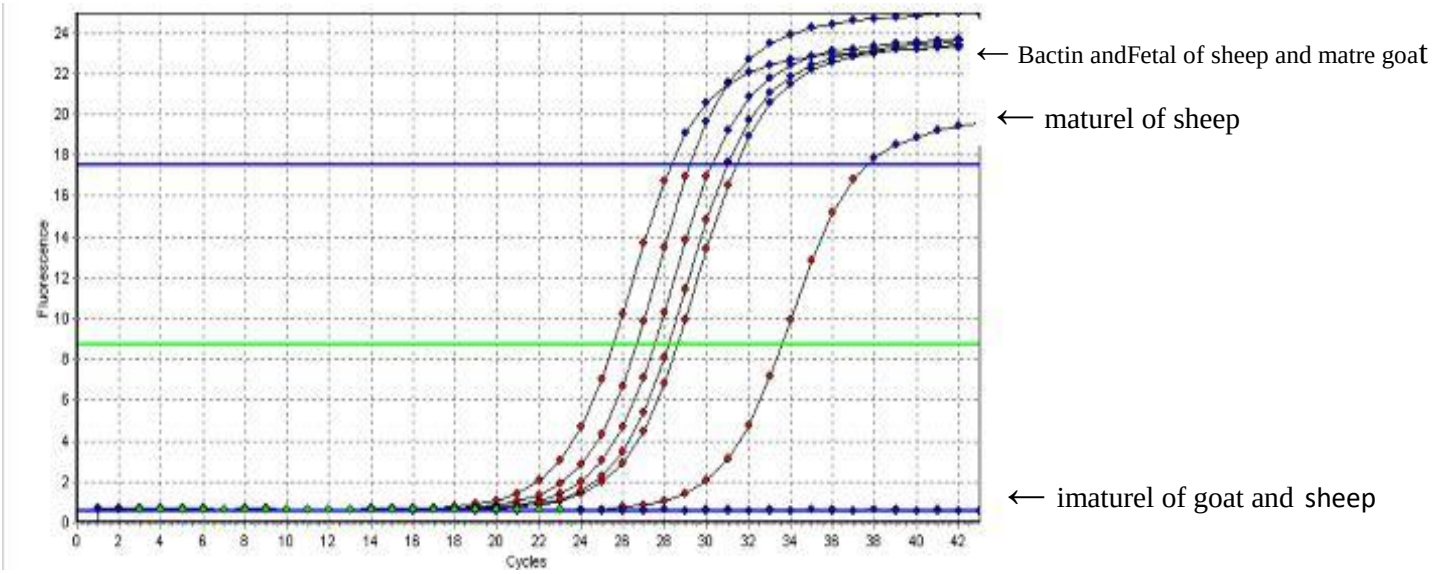

Figure 2. Curve of CD44 gene replication in immature oocytes, mature and fetal.

Table 3. Variance Analysis of CD44 gene expression in Baluchi, Tali sheep and Rayeni, Tali goat.

Sources changes	Mean-square	sum of squares	DF	F
Treatment	84.141	84.141	1	
Error	11.25	112.509	10	7.47
Total		2.8987	22	

DF= degrees of freedom; Minimum significance level = P <0.05.

point of the CD44 gene in all tissues of the animals was 62°C. Table 2 shows the thermal gradients of threshold cycler. By using Software line Reg PCR, the cycle curve shows the fluorescence light and CD44 expression in all tissues (Figure 2). CD44 gene in all tissues of sheep and goats were expressed in immature oocytes, but this expression in other tissues was indistinguishable. Rates of PCR efficiency were different in tissues of different animals. Expression levels in sheep and goat were different due to the significant difference in sheep and goat breeds, as shown in Table 3.

So we can conclude that CD44 expression is asignificant

Table 4.sheep, goat CD44 gene expression data analysis

Sources changes	Mean-square	sum of squares	DF	F
Treatment	8793	2.893	4	2616
Error	09017	222918	.	
Total		2.8987	22	

DF= degrees of freedom; Minimum significance level = P <0.05.

Table 5. Comparison of Average.

Sample	Goat Embryo	Sheep Embryo	Mature oocyte Tali goat	Mature oocyte Baluchi sheep	mature oocyte Rayeni goat
Sheep embryo	19.5 *	-	-	-	-
Mature oocyte Tali goat	1943 *	0984	-	-	-
Mature oocyte Baluchi sheep	.934 ***	7937 **	6.99	-	-
Mature oocyte Rayeni goat	20 ***	8904 **	6.66**	0.561	
Mature oocyte Kermani sheep	14.4***	11.5***	12.06***	5.971***	5.98**

test for comparison, which was done by examining the sample. In addition to the effect of race on expression levels of CD44, gene expression comparison between sheep and goats was done (Table 4). F that was obtained from Table 3 is higher than F in base table (P <0.05). So this test is more significant in the expression of these genes and also the obtained result was different between sheep and goat (Table 5) in the CD44 gene in comparison to the races at P <0.05 according to Duncan's method was studied.

Based on the table, it is clear that the greatest differences were found between goats' fetus and mature oocytes of sheep but minimum differences were found between Baluchi sheep mature oocytes and immature Rayeni goats' oocyte. Also, the greatest differences were found between mature oocytes of goats and Kermani sheep. From this table, we can conclude that CD44 gene expression in fetus of sheep and goats was higher than that of the other samples.

In this study, it is established for the first time that CD44 is expressed on mature oocytes and embryos of goat. Real time PCR was used to detect the expression of transcript. Similar result is reported in mature oocytes of additional mammalian class, like human (Toyokawa et al., 2005). Also we found that CD44 mRNA was not detected in immature oocytes. This is logical since immature oocytes need to get in touch with nearby granulose cells to allow nutrient passage and to get good development. This agrees with previous data on other mammalian class such as porcine (Campbell et al., 1995) and bovine (Luz et al., 2012), in which CD44 in immature oocytes was not detected.

It is now recognized that CD44 influences the growth of the cumulus cells throughout the oocyte maturation (oeplia et al., 2012), leading to fertility and excellence of oocytes (Luz et al., 2012). As the hyaluronan-CD44 communication is concerned in the introduction of meiotic recommence-ment, it was supposed that this receptor was expressed in the oocytes. However, it was established that this receptor is present only in cumulus cells, and not in the oocyte. New studies have shown that the meiotic maturation of oocytes is also a topic on regulation by the somatic section of the ovarian follicle (oplea et al., 2012). MPF that starts at the onset of meiotic recommencement is inhibited by intra-oocyte cAMP, which is transferred from cumulus cells via gap junctional communication inside COCs. Break of gap junctions in the COCs, which occurs in reply to the pre-ovulatory rush of gonadotropins (Assidi et al., 2008), leads to a drop in the intra-oocyte concentration of cAMP, followed by MPF activation and meiotic resumptions. The decrease of the intra-oocyte cAMP attentiveness was concealed by the inhibition of the interaction between hyaluronan and CD44. This result supports the concept that hyaluronan-CD44 interaction is concerned with the regulation of gap junctional communication and the termination of the cAMP flux from cumulus cells to oocytes (Yokoo et al., 2010).

No studies have established the role of the HA-CD44 system in oocyte maturation. However, one study (Luz et al., 2012) demonstrated that the squalor production of HA induced the phosphorylation of the CD44 receptor, most important for the start of kinase proteins, which are then translocated to nucleus. This flow is significant for mitogenic signal transduction and enough for the induction of cell propagation from the proto-oncogenic transcription factors (Daum et al., 1994). Since the mainconstituent in the extended cumulus is HA (Borg and Holland, 2008), this almost certainly explains the availability of the CD44 receptor in mature oocytes.

CD44 as well plays a role in embryo development up to

blastocyst phase (Kimura et al., 2007). In one trial (Oeplia et al., 2012), l mg/ml of HA was supplemented with the civilization medium; and the bovine embryos were established, which then developed to the blastocyst stage higher than when in a medium alone. These authors reported that the amalgamation of HA in a chemically defined medium obviously established the result of HA in the development of blastocyst configuration. This is in accord with the study of Miyano et al. (1994), who stated that the amount of degenerated porcine embryos were more inferior in the presence of HA than in its absence. It has been suggested that HA helps the development of embryos by regulating the action of factors that synthesize the embryo, in an autocrine way (Wheatley et al., 1993). Li et al. (2008) reported that at the beginning, production of HA occurs at about 18 h after the beginning of maturation. This is enthused by the growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15), which trigger hyaluron synthase enzyme expression, responsible for synthesis of HA. The best growth of refined COCs requires the presence of substrates of HA synthesis and a prolonged cumulus mass that might absolutely influence oocyte feasibility (Chen et al., 1993). HA shaped obviously by granulosa cells also stop fragmentation or segmentation of oocytes *in vitro* (Sato et al., 1994).

The presence of CD44 in mature oocytes and embryos suggests the expression of HA throughout maturation and development. The result of this study could be helpful in the description investigation and understanding of the physiological role of CD44 in the reproductive processes of the ovine class. Additional studies are necessary to elucidate the proceedings in which CD44 and HA are involved during maturation and embryo growth in goats and sheep.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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