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Original Article

Estimation of periostin as a biomarker for early pregnancy diagnosis in goats: a preliminary study

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Abstract

Background: Periostin (POSTN) is an extracellular matrix (ECM) protein that plays an important role in the metastatic process and cancer cell migration. As implantation is a similar mechanism to metastasis, it has been hypothesized that POSTN may also play a role in the implantation process. **Aims:** The aim of the present study was to compare POSTN and progesterone levels during the early pregnancy stage in Damascus goats. **Methods:** Forty goats were synchronized using progesterone based sponges and were mated upon estrus signs display. While ten goats were kept as control (CON) and were not allowed to mate. Blood samples were taken through jugular venepuncture from CON and synchronized goats on day 13, 15, 17, 19, and 21 of breeding. Progesterone and POSTN levels were determined by enzyme-linked immunosorbent assay (ELISA). Later the pregnancy diagnosis was confirmed by transabdominal ultrasonography on day 50 after mating. **Results:** Progesterone level was influenced by status of pregnancy and day of observation with an interaction between the status of pregnancy and day of observation in goats. Whereas POSTN level was only affected by the day of observation. **Conclusion:** POSTN level did not vary with progesterone level during phase of embryonic implantation in goats; however, standardization and application of different procedures for POSTN assay in a large group of animals might be useful as an early pregnancy biomarker in goats.

Key words: Biomarker, Goat, Periostin, Pregnancy, Progesterone

Introduction

The implantation process consists of a series of events that started from fusion of trophectoderm to the luminal epithelium of the uterus and this fusion subsequently turns into the placenta. There are various events like the release of the blastocyst from zona pellucida, attachment, and fusion of the blastocyst to the endometrium mammalian implantation process. Normally, this process is completed in three phases in ruminants: a) the blastocyst contacts the implantation site of the endometrium (apposition), b) trophoblast cells of the blastocyst attach to the receptive endometrial epithelium (adhesion), and c) invasive trophoblast cells cross the endometrial epithelial basement membrane and invade the endometrial stroma (invasion) (Bazer *et al.*, 2012; Erdem and Sarıbay, 2012).

The caprine conceptus growth during the pre-implantation period is similar as observed in bovine or ovine. Elongation of the embryo, enclosed in trophoblast, is a first prerequisite for implantation that is followed by superficial and non-invasive adhesions of trophectoderm-uterine epithelial cells. It resulted in strong apposition with no cellular damage to the uterine epithelium because of implantation; cotyledonary type placentation occurs in goats. Maternal recognition in goats occurs during 16-21 days of pregnancy which is brought about by conceptus to release the caprine IFNT (Bazer *et al.*, 2012).

The fusion of endometrium and embryo is the key factor for mutual and successful implantation and pregnancy. In addition, steroid hormones and immune cells related factors, blastocyst, and endometrium during implantation, stimulate significant changes in the superficial profiles of blastocyst, and endometrial extracellular matrix (ECM) proteins (Di Cello *et al.*, 2015). Extracellular matrix is an important regulator for normal connective tissue structure and function and is also essential for molecular signals to existing cell populations. Extracellular matrix consists of structural proteins (fibronectin, collagen, laminins, vitronectin, and periostin (POSTN)) and special proteins (proteoglycan, glycoproteins, growth factors, and matrix metalloproteinase). Extracellular matrix has a dynamic structure which alters in response to different stimuli such as mechanical, integrin signals or pathological conditions. Adhesion of cells to the ECM with integrin receptors; it regulates the shape, proliferation, intracellular stimulation and differentiation of cells, thus providing normal tissue function (Hamilton, 2008).

Periostin is secreted as an ECM protein and plays an important role in the metastatic process (Di Cello *et al.*, 2015). Periostin is a 90 kDa heparin-binding protein containing domains of 4 consecutive sequences of fasciline (fas 1) and a nitrogen end is bounded by a glycosylated disulfide bond (Hamilton, 2008). Periostin is present in the periodontal ligament and other connective tissues including tendons, skin, and bone. It is found in neoplastic tissues to a great extent and also found during cardiovascular diseases and the wound healing process (Hamilton, 2008; Di Cello *et al.*, 2015). Periostin plays a role in cancer cell migration and metastasis; it also regulates the interaction between cancer cells and the metastatic area. Particularly, its expression stimulates the first tumor cells to target metastatic tissues. The implantation process is similar to tumor cell metastasis in context to biological features like adhesion, proliferation, invasion, and epithelial-mesenchymal transition. The maternally recognized embryo targets the endometrial tissue for implantation and invasion in the attachment site as POSTN regulates cancer for metastasis (Di Cello *et al.*, 2015).

Morelli *et al.* (2014) defined the role of POSTN as an early serum biomarker in embryo quality, implantation of embryo, and determining of pregnancy. It was reported that a low level of serum POSTN is an indication of weak implantation and low POSTN concentration during the first trimester of pregnancy could lead to pregnancy loss. However, there is no previous report about the use of POSTN as an indicator of pregnancy establishment in goats. In this context, the present study aimed to determine the level of POSTN and progesterone in comparison during the implantation phase in the Damascus goats.

Materials and Methods

This study was conducted pursuant to approval numbered with 2017/1-2 of the Ethics Board of Hatay Mustafa Kemal University, Animal Experiments Local Ethics Committee.

Study area and husbandry

The study was carried out in a commercial goat enterprise in Nurdağı district of Gaziantep province. A total of forty (n=40) multiparous Damascus goats aged 3-5 years were included in this experiment. The goats were maintained on optimum feeding regimens with grazing. *Ad libitum* clean and freshwater access was provided for the experimental (EXP) animals.

Experimental design

Out of forty, thirty (EXP; n=30) were synchronized and the remaining 10 served as control (CON). The EXP goats were synchronized for estrus by placing the intravaginal sponges (Chronogest Cr, Intervet, Istanbul, Turkey) for 9 days. On the day of sponge removal, the goats were injected 550 IU pregnant mare serum gonadotropin (PMSG) (Chronogest/PMSG, 6000 IU, Intervet, Istanbul, Turkey) and 125 mcg d-cloprostenol (Senkrodin®, Vetaş, Turkey). The goats were mated upon displaying estrus signs. The mated goats in the synchronization group were considered as pregnant and non-mated goats in CON group were graded as non-pregnant. For measuring serum progesterone and POSTN estimation blood samples were taken from all the EXP goats on days 13, 15, 17, 19, and 21 from the vena jugularis by venipuncture to serum gel tubes and stored at -20°C until analysis. The mated goats were observed for pregnancy based on non-return rate and ultrasonography diagnosis. The samples were analysed for serum progesterone and POSTN estimation. Serum progesterone and POSTN estimation were done after kidding of the EXP goats. In the CON group, confirmed serum samples of non-pregnant goats were estimated for progesterone and POSTN. Plasma progesterone levels were analyzed by an electrochemiluminescent method in an autoanalyzer (Siemens Advia Centaur XP Immunoassay System, UK). Plasma POSTN concentrations were measured with enzyme-linked immunosorbent assay (ELISA) kit (Periostin Goat ELISA Kit/96 Test/Mybiosource/Cat.No:MBS101647) by following the instructions of the manufacturer. The manufacturer has determined the sensitivity of this kit as 0.1 µg/ml, no significant cross-reactivity or interference between this analyte and analogues is observed, and both intra-assay coefficient of variation (CV) (%) and inter-assay CV (%) are less than 15%.

Statistical analysis

Descriptive statistics are shown as arithmetic mean ± standard error. Variations in progesterone and POSTN levels during the study period among the EXP and CON groups were evaluated through two-way ANOVA repeated measurements. The relationship between progesterone and POSTN levels of the study and CON groups on days 13, 15, 17, 19, and 21 were analysed using the Pearson correlation coefficient (r). Statistical significance level was determined as $P<0.05$. All statistical analyzes were performed using SPSS 14.01 package program.

Results

The results of progesterone and POSTN estimation have been presented in Table 1. There was a significant effect of observation day and pregnancy status on the progesterone level in the EXP and CON groups at each day of sampling. A significant interaction of pregnancy status and day of observation for progesterone level was observed. In contrast, POSTN level was only affected by the day of observation. There was no interaction between pregnancy status and day of observation for POSTN level in goats. There were no positive or negative

Table 1: Serum progesterone (ng/ml) and POSTN concentrations (µg/ml) in non-pregnant (n=10) and pregnant (n=30) goats at different days of observations after mating

Variable	Status of animals	Sampling after insemination (day)					Mean	P-value		
		13	15	17	19	21		Day	Status	Day * status
Progesterone (ng/ml)	Pregnant	19.3 ±1.0[a, A]	17.7 ±0.9[b, A]	16.5 ±0.6[c, A]	16.5 ±0.5[c, A]	15.4 ±0.4[c, A]	17.1 ±0.6	<0.001	<0.001	0.049
	Nonpregnant	7.7 ±1.7[a, B]	6.0 ±1.5[a, B]	4.7 ±1.1[b, B]	1.6 ±0.9[c, B]	0.1 ±0.8[c, B]	4.0 ±1.0			
POSTN (µg/ml)	Pregnant	0.7 ±0.6[a]	0.7 ±0.1[a]	1.2 ±0.1[b]	1.1 ±0.1[b]	0.8 ±0.1[b]	0.9 ±0.1	<0.001	0.304	0.171
	Nonpregnant	0.7 ±0.1[a]	0.7 ±0.1[a]	1.3 ±0.2[b]	1.2 ±0.2[b]	1.3 ±0.2[b]	1.02 ±0.1			

Small letters ([a, b, c]) indicates the significant difference in rows and capital letters ([A, B]) shows the significance in columns. POSTN: Periostin

Table 2: Correlation between progesterone and POSTN in pregnant goats at different days of observations after mating

Day of sampling	r	P-value
13	0.290	0.120
15	-0.168	0.376
17	0.176	0.353
19	0.100	0.599
21	-0.187	0.323

r: Pearson correlation coefficient, and POSTN: Periostin

correlations between progesterone and POSTN levels in pregnant goats at any day of observation (days 13, 15, 17, 19, and 21) (Table 2).

Discussion

Early pregnancy diagnosis is challenging especially in small ruminants. Different methods are applied for pregnancy diagnosis which provides the accurate results after 40 days of breeding. Using transrectal ultrasonography, pregnancy diagnosis is done as early as 25 to 30 days of breeding; whereas it goes beyond 50 to 60 days when used in transabdominal ultrasonography. In sheep and goats, about 30-40% of fertilized ova died during the first three weeks of pregnancy and about 70-80% of these deaths occur with the first two weeks of insemination. Considering this situation, early pregnancy diagnosis by ultrasonography in goats is questionable. Measurements of progesterone for diagnosis of early pregnancy are also not an accurate way because early embryonic deaths, uterine or ovarian pathological conditions could misdiagnosis pregnancy status (Erdem and Sarıbay, 2012). Early pregnancy factors such as pregnancy-specific protein B (PSPB) and pregnancy glycoproteins also provide a better estimation with 100% accuracy in pregnancy diagnosis in sheep and goats after 21 days of breeding. However, there is a radioactive risk in determining serum analysis by radioimmunoassay (RIA) method (Erdem and Sarıbay, 2012).

Corpus luteum is the sole organ for progesterone in goats for the maintenance of pregnancy (Chao *et al.*, 2008; Erdem and Sarıbay, 2012). The progesterone level is nadir (0.1-0.5 ng/ml) at estrus time and rises to 2.5-3.5 ng/ml in pregnant or diestrous goats (Thorburn and Schneider, 1972; Thibier *et al.*, 1981). Different studies reported the progesterone level >1 ng to 4 ng/ml but its level peaks at 10-24 ng/ml during the phase of pregnancy in different goat breeds: Crossbred (Engeland *et al.*, 1997), Saanen and Alpine (Boscos *et al.*, 2003), Beetal (Khanum *et al.*, 2003), and Damascus (Al-Merestani *et al.*, 2003; Gaafar *et al.*, 2004). In the present study, the mean progesterone concentration in pregnant animals was 17.1 ng/ml during the early days of gestation in Damascus goats. In contrast, progesterone level in non-pregnant goats was 7.67 ng/ml on day 13 and the lowest level (0.11 ng/ml) was seen on day 21 that indicates the transition from luteal to the follicular phase. This variation suggested that ideal time is day 13 and 21 after mating if goats are diagnosed for early pregnancy based on progesterone concentrations.

POSTN plays a major role in cancer cell migration and metastasis and it also acts as a regulatory factor for the interaction of cancer cells and metastatic tissues. As implantation is a similar mechanism for cancer cell migration and metastasis, it has been assumed that POSTN might be involved in embryo implantation (Di Cello *et al.*, 2015). Previously, POSTN was detected in caruncular areas of human and mouse placenta on day 18 and 20 of pregnancy (Gillan *et al.*, 2002; Rios *et al.*, 2005) but its role in placentation has not been elucidated. Later, Savaris *et al.* (2008) reported a significant increase in POSTN amount with advancement of pregnancy stage. Moreover, it has been documented that the expression of POSTN is regulated by ovarian hormones and the stage of cycle (Hiroi *et al.*, 2008). It has also been reported that POSTN level significantly reduced in females when the pregnancy loss occurred spontaneously rather than the induced ones (Morelli *et al.*, 2014; Freis *et al.*, 2017). In contrast, POSTN level is almost undetectable in non-pregnant women compared to pregnant women. These reports confirm the relationship between pregnancy and POSTN expression but its exact mechanism is still unclear. Earlier, Ahn *et al.* (2009) identified the POSTN expression in sheep endometrium during the first three weeks of pregnancy and assumed that POSTN is associated with elongation and attachment of conceptus to the endometrium during early pregnancy. In this context, it was expected that pregnancy can be diagnosed during the implantation phase in goats through serum POSTN estimation. However, POSTN levels did not differ between pregnant and non-pregnant goats. Level of POSTN increased from the 13th to the 17th day of breeding in pregnant goats and decreased later on. These results do not support the report of Ahn *et al.* (2009) in sheep. Contradictory findings in present study might be due to species difference, method of POSTN measurement, or low concentration in peripheral circulation particularly during the phase of implantation. Contrary to women, it may be because of the non-invasive placenta in goats. In conclusion, the level of POSTN during the implantation phase was not different in pregnant and nonpregnant goats. As it is a preliminary study; therefore, a comparison of different methods for POSTN estimation including a large group of animals could reveal a better interpretation. Also, the measurement period may be extended to the late implantation process that needs to be evaluated in future aspects.

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Conflict of interest

The authors declare that they have no conflict of interest.

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