SERPINA 14 expression from endometrial epithelium in cows with embryonic mortality and aborted

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\textbf{Abstract}
In recent years, many studies have been conducted on SERPINA 14, which is one of the molecules suggested to have a role in the occurrence and continuation of pregnancy. In this study, it was aimed to detect the presence of SERPINA 14 mRNA by the reverse transcription-polymerase chain reaction (RT-PCR) in the endometrium of cows in which embryonic mortality and aborted had occurred. In this way, a novel approach can be developed towards embryonic mortality and abortion cases which lead to significant losses in cattle farming. For this purpose, endometrial biopsy samples were obtained from a total of 30 cows which were embryonic mortality (n=10), aborted (n=10) and normal pregnancy (n=10). The presence of SERPINA 14 mRNA in the samples was determined with RT-PCR. Consequently, SERPINA 14 mRNA was detected in the endometrial biopsy samples of all groups. Therefore, it was concluded that the role of SERPINA 14 in continuation of pregnancy could be related to its amount rather than its presence in cows and thereby the present study should be supported by further studies.

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\section*{1. Introduction}
Embryonic mortalities and abortions are important problems in animal breeding, especially dairy cattle breeding. Embryonic mortalities and abortions lead to great harm in the economy of cattle breeding. Be it due to infectious or non-infectious reasons, the ways these disorders develop are still being debated today. It is known that intrauterine mortality of the embryo and the fetus arises from interruption of mechanisms enabling a healthy continuation of pregnancy. The maternal immune system should function in a healthy way for occurrence, continuation and normal termination of pregnancy.

The maternal immune system tolerates the presence of paternal alloantigens without affecting the anti-infectious mechanisms during pregnancy. Thus, pregnancy is considered to continue through establishment of immune tolerance. Incomplete tolerance leads to abortions, embryonic mortalities and termination of pregnancy through causing
other gestational pathologies (Koch and Platt, 2007; Tafuri et al., 1995).

The uterine environment should be healthy for a successful fertility. The endometrium is locally prepared for transport of gametes and development of the embryo during the estrous cycle. The endometrium synthesizes and releases some molecules for continuation of pregnancy and for recognition by the mother. Uterine serpins/uterine milk proteins (serpin peptidase inhibitor, clade A [alpha-1 antiproteinase, antitrypsin], member 14, SERPINA14) are basic glycoproteins of the serpin super-family, which also includes serine peptidase inhibitors (serpins). SERPINA 14 molecules are major secretory proteins which were first described in the 1980s as ‘ovine uterine serpins’ in the sheep uterus, however, they were later determined to be synthesized in the endometrium of all ruminants during pregnancy (Bazer et al., 1979; Klein et al., 2006; Leslie and Hansen et al., 1991; Moffatt et al., 1987). Long term progesterone administration in sheep and cattle are known to cause stimulation of SERPINA 14 (Ing et al., 1989; Leslie et al., 1990; Moffatt et al., 1987). It was observed that long term administration of estradiol-17β and progesterone together in sheep and cattle did not stimulate SERPINA 14 excretion, however, administering progesterone alone caused a decrease in SERPINA 14 mRNA excretion (Spencer et al., 1999).

SERPINA 14 is known to contribute to the immunosuppressive effect of progesterone (Skopets et al., 1992). It has been suggested that SERPINA 14 inhibits lymphocyte functions in vitro and it has been reported to play a role in the development of maternal tolerance against the embryo (Hansen and Newton, 1988). However, it has been suggested that SERPINA 14 is not a predominant molecule in cows during pregnancy, but that it should be in significant amounts in the development of a histotrophic environment during pregnancy as in sheep (Segerson and Bazer, 1989). It was shown in vitro that activation of uterine NK cells is decreased by SERPINA 14 (Liu and Hansen, 1993, Tekin and Hansen, 2002), whereas it has no effect on proliferation of γδ T cells (these cells excrete significant cytokines for continuation of pregnancy) (Pelletier et al., 2000). It has been reported that SERPINA 14 prevents the negative events related to antibodies which develop against the embryo through making complexes with IgA and IgM (Hansen and Newton, 1988). Despite all these data, the immunomodulation mechanisms could not be fully explained as specific receptors of SERPINA 14 could not be detected yet.

1.1Objective of research

In the present study, it was aimed to determine the presence of SERPINA 14 mRNA by RT-PCR in the endometrium of cows with embryonic mortality and aborted.

2. Materials and Methods

2.1 Animals

This study was performed with a total of 30 cows from various breeds and at different ages with embryonic mortality (n=10), aborted (n=10) and normally pregnant cows (n=10). Cows with aborted were selected among the animals at different periods of pregnancy, were brought to Ruminant Clinic of Animal Hospital in Firat University, animals which had experienced embryonic mortality were selected from cattle breeding enterprises and normal pregnant cows were selected among animals in slaughterhouses in Elazig and the surroundings, which were going to be slaughtered without knowing that they were pregnant.

2.2 Determination of embryonic mortalities

Embryonic mortalities were determined according to the description by Prvanovic et al. (2009) with ultrasonography using a 5 or 7.5 MHz linear probe on the 17, 24, 35 and 45th days after artificial insemination had been performed.

2.3 Obtaining endometrial biopsy samples

The samples were obtained with sterile uterine biopsy catheters from the animals with aborted or embryonic mortality once every week. The endometrial samples of normal pregnant animals were obtained from the uterus of animals which were slaughtered in slaughterhouses. The obtained samples were placed into eppendorf tubes without being subjected to any procedure and stored at -80° C until the analyses were carried out.

2.4 RNA extraction

The samples which were collected and stored at -80° C were kept at +4° C for one night to dissolve. About 50 µg of the tissue samples were transferred into eppendorf tubes. RNA isolations were done using a commercial RNA isolation kit (ZR RNA mini prep. Catalogue no: R 1065). For RNA elution, approximately 30-40 µl of RNAase-DNAase-free water was added onto the RNA isolation column and kept at room temperature for 2 minutes. The samples were centrifuged and RNA was obtained.

2.5 Reverse transcription

The reverse transcription (RT) procedure was performed in order to obtain cDNA in RNA samples. In this study, the amplification of the 126-base-pair (bp) part of SERPINA 14 gene was aimed. Forward: 5’-ATATCATCTTCTCCCCATGG-3’ and Reverse: 5’-GTGCACATCCACAGTTTGG-3’ primers
were used to replicate the gene region. For control the success of RT and PCR stages RNA extraction in addition, the RT and PCR stages were realized with primers (Forward:5’-AAGTCTTTCGGTCCGGG-3’ and Reverse:5’-GGACATCTAAGGGCATCACA-3’) for amplification of 18S ribosomal RNAs in all cells. Sterile dH2O was used as negative control samples.

2.6 PCR
The PCR stage was realized using cDNA templates obtained from the RT stage. While reverse primers were used in RT stages, forward and reverse primers were used together in the PCR stage.

2.7 Agarose gel electrophoresis
1.5% agarose gel was prepared in order to visualize the amplification products following the RT-PCR stage. The samples loaded onto the wells were eluted for about 1 hour and 30 minutes at approximately 100 volts. The electrophoresis results were evaluated under UV light.

2.8 Statistical analysis
A statistical calculation was not performed due to similar SERPINA 14 mRNAs having been detected on endometrial biopsy samples obtained from all groups.

3. Results and Discussion
According to the electrophoresis results, approximately 365 base pair (bp) of amplification product was detected with 18S rRNA primers from tissues of all groups included in the study (pregnant, aborted, early embryonic mortality) by RT-PCR. Using RNAs obtained from tissue samples and SERPINA 14 specific primers, approximately 126 bp of amplification product was detected in all tissue samples in agarose gel (Figure 1).

SERPINA 14 is known to be excreted from the endometrium during pregnancy and the estrous cycle in pigs, sheep and cows, and this is known to be regulated by estradiol and progesterone (Bauersachs et al., 2005; Leslie et al., 1999; Padua and Hansen, 2010). It is known that a great amount of SERPINA 14 is excreted from the endometrium of pregnant cows (Moffatt et al., 1987), which prevents fetal rejection through inhibiting the lymphocyte proliferation (Skopets et al., 1992) and cytotoxic activity of NK cells (Liu and Hansen, 1993). This molecule is reported to stabilize uteroferritin, an iron-binding protein, in pigs during pregnancy and transports iron to the fetus (Padua and Hansen, 2010). In cows, SERPINA 14 is known to be low in the estrous phase of the cycle, increase in mid-luteal phase, and reach its
maximum level during pregnancy (Ulbrich et al., 2009). While SERPINA 14 secretion had been reported to have been regulated by only progesterone previously, it was suggested thereafter that it was regulated by estradiol in the estrous cycle and by progesterone in the luteal phase (Leslie and Hansen, 1991).

In the early periods of pregnancy, progesterone suppresses the immune functions of the uterus by stimulating the endometrial secretion of SERPINA 14 and thereby contributing to the occurrence and continuation of pregnancy (Hansen, 1998). SERPINA 14 mRNAs is up-regulated on the 18th day of pregnancy in cows (Klein et al., 2006). Progesterone, prolactin, placental lactogen, growth hormone and IFNT have significant effects on the expression of SERPINA 14 from the endometrium (Leslie and Hansen, 1991; Moffatt et al., 1987; Spencer and Bazer, 2002; Stewart et al., 2000).

SERPINA 14 has also been detected in uterine fluid in the later periods of pregnancy in cows and sheep (Padua and Hansen, 2010).

Conclusion

Despite all literature searches, no studies could be found about endometrial expression of SERPINA 14 in cows with aborted and embryonic mortality. In the present study, transcription of SERPINA 14 RNA was determined in all endometrial samples obtained from cows in which aborted and early embryonic mortality had occurred and which were pregnant. Thus, no difference was found between the groups for the presence of SERPINA 14 RNA. The RT-PCR assay has sufficient sensitivity to detect the small number of targeted RNA. Hence, in this study, detection of SERPINA 14 RNAs in all samples does not provide data about the amount of SERPINA 14 RNA. We consider that the amount, rather than the presence of SERPINA 14 RNA is important for continuation of pregnancy. Therefore, it was concluded that determination of the amount of SERPINA 14 will be more beneficial than questioning the presence of SERPINA 14 in related tissues.

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Authors’ Contribution and Competing Interests

All authors have read the manuscript and do not have any conflicting of interests.


