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Biochemical Insights into Caprine Reproduction: MMP Analysis Using Gelatin Zymography

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ABSTRACT: A comparative study was conducted to find out the existence of gelatinases in the serum of native goat breed of Tamil Nadu 'Salem black'. Experimental animals were divided into two groups for each breed *viz.*, Group I (Pregnant), Group II (Non Pregnant). In each group, the serum samples were collected from twelve healthy animals in a heparinized vacutainer, during early morning before feeding the animals. All the serum samples were subjected to gelatin zymography. It was confirmed that the major bands were observed at 220, 135 and 92 kDa of MMP-9 and 72 kDa of MMP-2 in both the groups. In group I, two prominent bands were observed at 92 kDa and 72 kDa and it represents the latent forms of MMP-9 and MMP-2 respectively. Additionally, three lytic bands representing the proforms of MMP-9 were detected in each group at 220, 135 and 125 kDa. In comparison to the non-pregnant group, the pregnant groups' latent form of MMP-2 (72 kDa) intensity was 1.5 times greater. Likewise, the pregnant group had increased MMP-2 expression than the non-pregnant group. MMP-2 levels were higher in the early stages of pregnancy than in the nonpregnant group. Nonetheless, the MMP-9 intensity remained constant and increased during the middle to late phases of management. MMP-9 involved in tissue remodelling, which was more noticeable in the late gestational phase. With possible ramifications for reproductive control and early pregnancy diagnoses in Salem Black goats, these results lend credence to the theory that MMP-9 is a crucial biomarker of pregnancy-related ECM remodeling in ruminants.

Keywords: Gelatin zymography, MMP-2, MMP-9, Gelatinases, Bands.

INTRODUCTION

Goat production in India is an important part of the agricultural economy, particularly in rural areas, where goats serve as a source of food, income, and livelihood. Goats are hardy animals, requiring minimal resources, and are particularly suited to India's diverse climatic conditions. Tamil Nadu, one of the major states in India, plays a significant role in goat farming due to its favorable climate and agricultural practices. Tamil Nadu has a significant population of goats, contributing notably to the overall goat population in India. According to the 20th Livestock Census (2019), the total goat population in Tamil Nadu is over 17 million, making it one of the leading states in goat farming in India. The Salem Black goat is a registered native breed of Tamil Nadu, highly valued for its unique black coat, high milk production, and adaptability to tropical climates. As a crucial part of rural livelihoods, these goats significantly contribute to the agricultural economy. Reproductive efficiency is a critical factor influencing their productivity; thus, understanding the physiological mechanisms behind their reproductive cycle is essential for improving breeding strategies. Matrix metalloproteinases (MMPs) are zinc-dependent enzymes that degrade extracellular matrix (ECM) proteins (Nagase *et al.*, 2006). MMP-2 and MMP-9 play a vital role in tissue remodeling, inflammation, wound healing, and possibly disease progression in goats. Their serum levels can be a valuable marker for assessing various physiological and pathological conditions, making them important for veterinary diagnosis, disease monitoring, and understanding the underlying mechanisms of tissue repair and inflammation in goats. Research on goat breeds is still scarce, despite the fact that MMPs in domestic animals

such as sheep, cattle, and buffalo have been the subject of several studies. This study aimed to investigate the gelatin zymography profile of pregnant and non-pregnant Salem Black goats, shedding light on the role of MMPs in their reproductive cycle.

MATERIALS AND METHODS

This study was conducted at the Department of Veterinary Biochemistry, TANUVAS-Veterinary College and Research Institute, Salem, Tamil Nadu, India.

Twenty four healthy Salem Black goats were chosen, divided into two groups (Group I Pregnant; n = 12) and (Group II non pregnant; n = 12). Before the animals were fed in the morning, blood samples from each animal were taken in a heparinized vacutainer. After centrifuging the blood samples for 15 minutes at 3000 rpm, the separated serum was examined using a spectrophotometer to determine its protein concentration using photometric assessment of blue color. Using different concentrations of bovine serum albumin (BSA) as a standard, the standard curve was constructed. For subsequent examination, the serum samples were kept at -20°C.

Gelatin Zymography. Following the addition of a co-polymerizing gelatin substrate (0.3%), the serum samples were subjected to modified SDS-PAGE (a modification of Laemmli's method performed by Heussen and Dowdle (1980)). Subsequent procedures were conducted using the methodology outlined from our laboratory (Prakash Krupakaran *et al*., 2016). Human capillary blood gelatinase was utilized as the standard marker for comparing the zymogram bands as per the approach carried out by Makowski and Ramsby (1996), and further procedures were given by Prakash Krupakaran *et al*. (2016).

RESULTS AND DISCUSSION

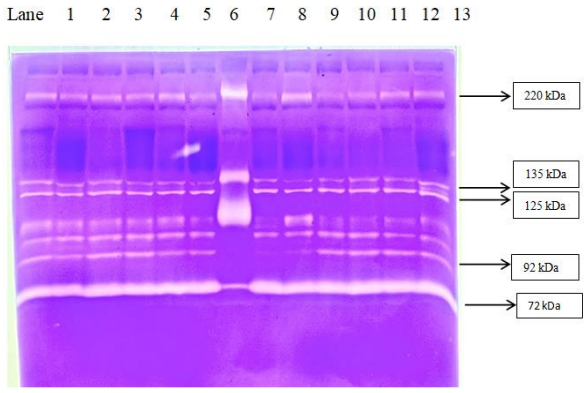
The gelatin zymography analysis of serum samples from pregnant and non-pregnant Salem Black breed goats are presented in Fig. 1, revealed distinct MMP activity profiles, highlighting variations in gelatinase expression based on reproductive status. Since every serum sample totally broke down the gelatin, they were all proteolytically active. It was verified that the main bands were seen in both groups at 72 kDa for MMP-2 and 220, 135 and 92 kDa for MMP-9. The latent forms of MMP-9 and MMP-2 are represented by the two significant bands that were seen in G I (P) goats at 92 kDa and 72 kDa, respectively. Additionally, two lytic bands representing the proforms of MMP-9 were detected in each group at 220 and 135 kDa. Nevertheless, neither MMP-9 nor MMP-2 is seen in their active forms. Likewise, in the non-pregnant group (G II), the latent forms of MMP-9 (92 kDa) and MMP-2 (72 kDa) were found. Similarly, MMP-9 proforms were also detected at the 220 and 135 kDa levels in every cohort. In both groups, MMP-2 (72 kDa) was significantly more prevalent than the human marker (lane 7).

Expression of MMP-9 in Pregnant and Non-pregnant Salem Black Goats. In order to determine the influence of pregnancy on the expression of gelatinase, the gelatin zymograms of the two groups were examined. In Pregnant Group I (Lanes 1-6), strong bands observed at 72 kDa, indicating high MMP-2 enzymatic activity. The pro forms of MMP-9 also observed at 220, 135, 125 kDa indicating the activity of MMP-9 was higher than G II. Further, faint pro-MMP-9 bands (111 kDa) observed in some samples, suggesting latent enzyme activation. Overall, the intensity suggests that MMP-9 is highly up regulated during pregnancy. However, in non-pregnant Group II (Lanes 8-13), weaker bands at 92 kDa, suggesting lower MMP-9 activity compared to the pregnant group and Minimal pro-MMP-9 bands, indicating that enzyme activation is limited in non-pregnant goats. Two to three times as much intensity was present in the latent form of MMP-2 (72 kDa) as in the latent form of MMP-9 (92 kDa). Our findings concurred with those of Prakash Krupakaran *et al*. (2015); Sanannam *et al*. (2024). The earlier study by Krupakaran *et al*. (2015), observed that pregnant buffaloes had significant bands at 220, 92, and 72 kDa. In comparison to non-pregnant buffaloes, the 92kDa MMP-9 band was highly noticeable, and its activity was three times more than that of MMP-2. Similarly, these results were in consistent with those of Riley *et al*. (1999); Novera *et al*. (2002). Riley *et al*. (1999) demonstrated that pregnant women have gelatinase activity, with the latent form of MMP-2 (72 kDa) being the predominant gelatinase. Similarly, Sanannam *et al*. (2024) investigated the MMP-9 expression levels in pregnant and non-pregnant pigs and discovered that pregnant pigs had significantly greater MMP-9 levels than their non-pregnant counterparts. Nonetheless, there was no discernible difference in the MMP-2 levels between pregnant and non-pregnant pigs. MMP-9 plays a crucial role in uterine remodeling, trophoblast invasion, and placental development (Curry and Osteen 2003). A significant higher MMP-2 activity was observed in the endometrium and myometrium of pregnant groups compared with the non-pregnant group. Pregnant uterus produces a significant higher amount of activated MMPs compared with the non-pregnant uterus. Highest MMP-2 and MMP-9 activities were reached around the time of implantation. The increased MMP-9 activity in pregnant goats aligns with its role in ECM degradation, facilitating vascularization and fetal development. The strong MMP-9 bands in pregnant goats suggest that this enzyme is actively breaking down ECM proteins, promoting placental invasion and uterine expansion. The lower MMP-9 expression in non-pregnant goats indicates that ECM remodeling is minimal when pregnancy-related changes are absent. Similar patterns have been reported in cattle and sheep, where MMP-9 expression is highest in mid to late pregnancy (Kizaki *et al*., 2008; Kim *et al*., 2018). MMP-9 helps remodel blood vessels, ensuring proper nutrient and oxygen supply to the fetus. Higher expression in pregnant goats indicates active vascularization supporting fetal growth. Pregnancy is associated with high progesterone and estrogen levels, which up regulate MMP-9 expression. In contrast, in non-pregnant animals, MMP-9 remains suppressed as

ECM turnover is minimal. Lower levels of MMP-9 indicate less ECM remodeling, as no placental invasion or tissue expansion occurs. MMP-9 activity is primarily regulated by estrous cycle changes, showing minor fluctuations but remaining significantly lower than in pregnant goats.

Expression of MMP-2 in Pregnant and Non-pregnant Salem Black Goats. MMP-2 (gelatinase A) is another key enzyme involved in extracellular matrix (ECM) degradation and reproductive tissue remodeling. In G I (Lanes 1-6), the intensity of latent form of MMP-2 (72 kDa) was 1.5 times higher than the non-pregnant groups (G II: lane 8-13). However, the active form of MMP-2 was not present in both the groups. Our findings concurred with those of Balamurugan *et al.* (2017); Balamurugan *et al*. (2020). The higher active MMP-2 levels in pregnant goats suggest its role in maintaining endometrial integrity and facilitating blood vessel formation. The lower MMP-2 activity in non-pregnant goats, along with the presence of pro-MMP-2 bands, indicates that ECM remodeling is reduced in the absence of pregnancy. Unlike MMP-9, which was highly up regulated, MMP-2 had a less dramatic difference in expression levels. MMP-2 functions in normal ECM turnover but plays a secondary role in pregnancy compared to MMP-9. This finding aligns with studies in sheep and cattle, where MMP-2 showed moderate increases during pregnancy but remained less variable than MMP-9 (Curry and Osteen 2003). According to human research, MMP-2 has a crucial role in the early stages of trophoblast invasion (6–8 weeks), while MMP-2 and MMP-9 both play a substantial role in the later stages (9–12 weeks) (Staun-Ram, *et al*., 2004). By dissolving collagen and extracellular matrix, MMP-2 and MMP-9 facilitate trophoblast invasion and enhance embryo implantation (Kim *et al*., 2011). It was concluded that MMP-2 was detected in early stages of pregnancy and MMP-2 was detected in mid stage and continue to increase in later stages of pregnancy.

Gelatin zymography of Matrix Metallo Proteinases in serum samples of pregnant and non-pregnant female Salem Black breed goats



Lane 1 to 6 – Pregnant Salem black breed goats serum samples

Lane 8 to 13 – non -pregnant Salem black breed goats serum samples

Lane 7 - Human capillary blood gelatinases as marker

Fig. 1. Gelatin zymography of Matrix Metallo Proteinases in serum samples of female Salem Black breed goats (13 microliters of sample Loaded in each well).

CONCLUSIONS

It was concluded that the expression of gelatinase activity was confirmed in both the groups. During early stages of pregnancy, the expression of MMP 2 was higher in pregnant groups than non-pregnant groups. As pregnancy advances, the expression of MMP-9 was higher in pregnant groups than nonpregnant groups. Since, MMP-9 was involved in tissue remodeling during advance stages of pregnancy, it was expressed at higher concentration. MMP-9 expression is significantly up regulated in pregnant Salem Black goats, reflecting its essential role in trophoblast invasion, ECM degradation, and placental vascularization. The lower expression in non-pregnant goats suggests that MMP-9 is primarily involved in pregnancy-associated uterine changes. In contrast, MMP-2 was expressed in both groups, albeit with a moderate increase in pregnancy, indicating its role in general uterine tissue remodeling rather than active placental invasion. These findings support the hypothesis that MMP-9 serves as a key biomarker of pregnancy-related ECM remodeling in ruminants, with potential implications for reproductive management and early pregnancy diagnostics in Salem Black goats.

FUTURE SCOPE

Further studies could be done by investigating the relationship between MMP activity and reproductive hormones such as progesterone, estrogen, and relaxin. Extending the study to uterine, placental, and ovarian tissues to determine localized MMP activity. Expanding the research to other native and commercial goat breeds to assess breed-specific differences in MMP expression. Exploring the potential of MMP-9 as a biomarker for pregnancy detection in goats.

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Conflict of Interest. None.

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