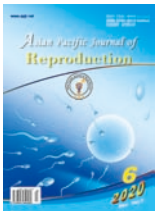

Review Article

Asian Pacific Journal of Reproduction

Journal homepage: www.apjr.net

doi: 10.4103/2305-0500.298772

Pregnancy-associated glycoproteins as a potential marker for diagnosis of early pregnancy in goats: A scoping reviewing

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ABSTRACT

Early diagnosis of pregnancy plays an important role to minimize reproductive losses in farm animals. There are several methods for pregnancy diagnosis like profiling of reproductive hormones (such as progesterone and estrone sulfate), but sometimes they provide false-positive results. Embryo specific pregnancy markers, which delineate the presence and viability of the embryo, are considered as perfect for pregnancy determination. Pregnancy-associated glycoproteins are distinguished as the best indicator for the determination of early pregnancy, fetal number, and birth weight of kids. Pregnancy-associated glycoproteins are structurally correlated to aspartic proteinase and are communicated in the external epithelial cell layer of the placenta. They have been found to share about half amino acid sequence identity with pepsinogen, pepsin, cathepsin D and E. Dislike different individuals from aspartic proteinase family, numerous pregnancy-associated glycoproteins appear to be latent compound as a result of amino acid substitutions in and around the catalytic site. This review is to discuss the scope and prospects of pregnancy-associated glycoproteins as a pregnancy marker in farm animals, more specifically in goats.

KEYWORDS: Pregnancy-associated glycoproteins; Early pregnancy; Farm animals; Placenta; Goats

1. Introduction

The goat is one of the important rising domesticated animal species all over the world because of their incredible adaptability to fluctuating natural conditions and nutritional regime. The present goat population in India is 132.74 million, which accounts for around 12.69% of the world's goat population. However, goats contribute only about 3.24% of the total milk production in India[1]. Lower reproductive performance is the explanation behind this production inadequacy. The early and precise pregnancy analysis can be a useful strategy for checking reproductive performance and consequently improve the reproductive proficiency of goat groups, as this allows timely breeding or culling of non-pregnant animals. Several methods such as abdominal palpation, radiography, ultrasonography, and detection of pregnancy-related hormones are being used for pregnancy detection in small ruminants with variable diagnostic accuracies[2], but these methods are less reliable for pregnancy detection as compared with determination of pregnancy-associated glycoprotein (PAG).

Pregnancy is established and maintained in response to interactions among the conceptus, ovary, and uterus and these interactions are continued by two-route communication between mother and conceptus (embryo and extraembryonic layer). These connections between mother and conceptus are seen as potential markers for placental remodeling, pregnancy recognition, and successful implantation[3]. Different particles that incorporate steroid hormones, peptide hormones, and cytokines and development factors are obligated for these significant signs to the maternal system to support a pregnancy[4,5]. Substances, which are derivatives of conceptus, are believed to be specific and reliable markers of pregnancy and fetal well-being[6].

The PAG is considered as a more suitable biomarker for pregnancy in goats since PAG solely is of placental origin in ruminants[7]. Nonetheless, the PAG profile during pregnancy can vary depending

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How to cite this article: Sharma N, Singh SP, Bharadwaj A, Natesan R. Pregnancy-associated glycoproteins as a potential marker for diagnosis of early pregnancy in goats: A scoping reviewing. *Asian Pac J Reprod* 2020; 9(6): 255-260.

Article history: Received: 16 July 2020; Revision: 21 September 2020; Accepted: 30 September 2020; Available online: 1 November 2020

on different factors including stage of gestation[8], breed of animal[9], and the type of assay used[10]. Thus, due to the possibility of variation in milk and plasma PAG levels, it is important to investigate standard profile of PAG in different biological samples before relying on a particular assay for pregnancy diagnosis in goats. This review is tothrow some light on PAG, primarily those concerned with its characteristics and physiological functions in goats.

2. Isolation and characterization

The placenta produces numerous proteins and it has been accounted for a long time in various species. A few models are human chorionic gonadotropin, equine chorionic gonadotropin, and interferon-tau (discovered in ruminants as a pregnancy recognition signal and originally named ovine trophoblast protein-1)[11]. Proteins that are released by the placenta, when recognized in the peripheral circulation of the mother, are considered important markers for both pregnancy and fetal well-being[12]. In 1982, two pregnancy-specific proteins (PSPA and PSPB) were isolated from the bovine placental membrane by Barbato *et al*[13]. Subsequently, PSPA was distinguished as a fetoprotein, which is not entirely restricted to pregnancy, while PSPB was affirmed as placenta-specific protein. In another research, Zoli *et al*[14] isolated a PAG (later assigned bPAG1) from bovine fetal cotyledons. It has different isoforms with a molecular weight of 67 kDa. Afterward, different studies confirmed that PSPB and bPAG1 were closely related in their primary structure[15]. In another research, glycoproteins which were immunologically related to bPAG1and PSPB have been also secluded from ovine fetal cotyledons. Later, they were designated as oPSPB, which were also detected in maternal blood after 3 weeks of breeding. These glycoproteins (either PSPB or PAG) have been used as a pregnancy marker in farm animals[16]. In cattle[17], ewe[18], goat[12], buffalo[19] and bison[20], PAGs were isolated from cotyledon by different biochemical procedures.

Isolation of the cDNAs, coding for bPAG1 and oPAG1 demonstrated that they had a place with the aspartic proteinase gene family, with more than half amino acid arrangement character to pepsin, cathepsin D, and cathepsin E and roughly 73% characteristics to each other. Recent studies have revealed that different PAG cDNAs are not expressed throughout pregnancy[16]. Some are expressed early while others are expressed only when pregnancy progresses[21]. Substitution of critical amino acids in the active site region rendered enzymatic inactivation to the PAGs[12] (Table 1). The PAGs are the results of trophoblast binucleate cells, which combine with uterine epithelial cells at implantation locale[14].

3. Classification of PAGs

As per placental tissue RNA library screening, which has been made by Garbayo *et al*[26] in goats, 11 different transcripts have been reported. These 11 transcripts have been categorized into two different classes, named as "modern PAG" (PAG- Ⅰ subgroup) and "ancient PAG" (PAG- Ⅱ subgroup). Ancient PAG includes PAG-2 and PAG-8 and is expressed by all types of trophoblast cells (both mono and binucleate trophoblastic cells) and the second group *i.e*. modern PAG contains PAG-1, PAG-3, PAG-4, PAG-6, PAG-7, PAG-9, PAG-10 and PAG-11 and these PAG members are expressed exclusively by specific binucleate cells[24] and exhibited more than 80% sequence identity with each other.

4. Methods of PAGs determination

Determination of PAG in maternal blood has served as a useful tool for pregnancy diagnosis in ruminants. Different PAG isoforms are detectable in the peripheral blood as early as the 4th week of pregnancy in goats and cattle by using different measurement techniques such as radioimmunoassay[27] and enzyme-linked immunosorbent assays (ELISA). Different homologous and heterologous immunoassays have been established for determining PAG concentration in cattle[28,29], sheep[18] and goats[27].

Table 1. Different pregnancy-associated glycoproteins isolated from placental tissues of different species.

Name	Molecular weight (kDa)	pH	Day of pregnancy	Species	Reference
cPAG-1	62, 59, 55	4.9-6.2	48-69	Goat	[12]
bPAG-1	67	4.4-5.4	90	Cattle	[13]
zebuPAG-1	51-69	4.4-6.7	70	Zebu cattle	[17]
bubPAG	52-77	-	Mid late pregnancy	Buffalo	[19]
EbPAG	50-71	3.7-7.4	45-120	Europian bison	[20]
oPAG-1	47-90	4.1-5.9	15-25	Sheep	[22]
pPAG-1	42-79	-	15-77	Pig	[23]
ePAG	37, 41	4.8-6.2	15	Horse	[24]
AmPAG	56-75	4.0-4.6	120	American bison	[25]

-: Non; PAG: Pregnancy-associated glycoprotein.

5. Factors influencing PAG concentration throughout pregnancy

In the recent 20 years, diverse studies have been done to investigate factors that may influence the PAG concentration throughout pregnancy in ruminants. In addition to the rise in PAG concentration throughout the pregnancy, as observed in the previous studies[29-31], other affecting factors like fetal number, body weight of kids and gender of kids, have also been revealed. As outlined by Shahin *et al*[9] and Patel *et al*[31], fetal number was observed to affect the PAG concentration positively. In goats, it has been proved that the increased number of fetuses could affect the PAG concentration throughout the pregnancy. The effect of fetal number on plasma concentrations of PAG was first observed during early pregnancy that continued throughout mid-gestation[32]. During mid-pregnancy, does bearing twin fetuses have a greater mean plasma concentration of all PAG. Overall, fetal number and birth weight of kids affected the plasma profile of PAG. Plasma PAG was identified as the best predictor for fetal number and birth weight of kids. Furthermore, day 58 of gestation was found to be the most appropriate single time point of blood sampling for prediction of kidding size using plasma PAG as a biomarker in goats[33].

It has been demonstrated that fetal gender has a significant influence on the PAG levels in farm animals during different stages of pregnancy. Ranilla *et al*[34] reported that ewes with male fetuses have a higher concentration of PAG level in comparison to those with female ones, whileWallace *et al*[35] stated that there are no significant differences in the PSPB levels between male and female singleton fetuses. Additionally, Lopez-Gatiuset *et al*[36] revealed no significant effect of fetal sex on the PAG concentration, which was inconsistentwith the outcomes reported by Serrano *et al*[37]. According to different studies, there are some indications that animals with *in vitro* fertilization have significant differences in the PAG concentration[38,39]. Ayad *et al* observed that progesterone concentration affects the PAG level positively[40] in farm animals during the first trimester of pregnancy. On the contrary, according to Lopez-Gatius *et al*[41], there is no significant relation between progesterone and PAG.

6. Temporal change in concentration of PAGs

There are varieties in transient expression patterns of PAGs. For instance, some PAGs are expressed relatively early in the conception, while other PAGs do not show up until later in pregnancy[14,21]. Individual PAGs are communicated at specific stages and absent at others[21]. On the whole, the expression of the members of the PAG-Ⅱ group starts earlier and stays longer than PAG-Ⅰ group. Up to now, different cDNAs have been recognized in farm animals. Different caprine cDNAs are differentiated and they are different by at least 5% in nucleotide sequence identity[26]. The PAG transcripts, those identified with caPAG2 and caPAG8, were the earliest to be communicated. Their mRNA was visible on day 18 of gestation. However, transcripts that hybridized to the caPAG8 were communicated all through pregnancy, except for day 24 of gestation. In addition, transcripts for most of the PAG-Ⅰ group were communicated significantly ahead just from day 45 of gestation with the exception of caPAG-4, which is the only element of PAG-Ⅰ, whose expression could be earlier. For instance, on days 25 and 30 of gestation, however, it was significant at a lower level. The concentration of PAG decreased steadily throughout the postpartum period until became untraceable about 100 days of postpartum[17]. The caPAG profile was characterised by a rapid increase during early gestation (day 28-day 51), ranging from 1.21±0.18 to 2.65±0.19 (mean±standard deviation) and attainment of the greatest concentration (2.95±0.21) on day 58 of pregnancy. The concentration of caPAG remained at this relatively greater value until the last observation before the day of parturition (*i.e*., day 135).

7. Physiological role of PAGs

PAG is considered as a good indicator of embryo viability, as it has been associated to be luteotrophic and luteoprotective. PAG proteins were evaluated to have a local immunosuppressive effect that can be included in the maintenance of the histocompatible feto-maternal unit. PAG enhances the secretion of alpha chemokine, which is suggested to play a role in mediating different actions like adhesion, inflammation, and angiogenesis associated with the implantation of the embryo. In spite of the diverse studies and theories about their functions, the real functions of the PAGs are still not known. The high homology of the residues within a domain, which creates an active site (substrate-binding cleft) of the PAG proteins, is likely essential for some function of these molecules. In ruminants, luteal cells treated with the bPSPB augmented generation of PGF2α and PGE2, without influence on progesterone secretion[42]. Conversely, another study illustrated that PSPB treatment expanded progesterone secretion by mixed little and extensive (days 17-18) bovine luteal cells[12].

8. Application of PAGs in diagnosing and monitoring pregnancy

Recently, determination of the PAG in maternal blood has served as a valuable tool for pregnancy determination in ruminants. Diverse PAG isoforms are recognizable in the peripheral blood of goats and cattle by utilizing various estimation methods, for example,

radioimmunoassay[27] and ELISA[28].

Determination of PAG helps diagnose pregnancy, because any unsettling in the fetal status, *i.e.* fetal death, will bring about a disturbance in the placental function and the expression of placental yields, for example, PAG. If there is an occurrence of fetal mortality, the concentration of PAG will fall rapidly underneath the PAG level which was recorded in the normal pregnant animal at the same stage of pregnancy[43,44], since the number of embryos carrying by the mother affects the PAG concentration, in cows[31], sheep[30] and goats[45,27].

The early pregnancy detection in goats based on PAG concentration in whole and skim milk was accurate between days 26 and 51 post-breeding. Day 37 post-mating was identified as the best suitable time point for pregnancy diagnosis by using milk samples in goats[46]. A strong positive correlation coupled with high Kappa value indicates suitability of both whole and skim milk samples for PAG estimation and early pregnancy diagnosis in goats. Further investigation into the association of milk PAG with early embryonic mortality may provide a valuable tool to study the functioning of fetoplacental unit in goats[46].

8.1. Pregnancy diagnosis in farm animals

In veterinary practice, pregnancy confirmation and trophoblastic functions are determined by the estimation of the PAG group in peripheral maternal blood circulation. However, considerable amounts of PAG have been additionally found in around 20% of virgin heifers and non-pregnant cows and have been demonstrated by radioimmunoassay system. An ELISA test for PSPB (BioPRYN™, BioTracking, Moscow, ID, USA) was made available and being prescribed for pregnancy diagnosis from day 30 after mating in farm animals[18].

8.2. Pregnancy diagnosis in ewes

In ewes, pregnancy diagnosis by PAG-radioimmunoassay can be performed in both plasma[47,48], and milk samples[18]. At farm conditions, the measurement of PAG concentration is the most reliable method for pregnancy diagnosis. Determination of plasmatic concentration of PAG/PSPB particles in ewes can give helpful data to create proper nourishing methodologies for pregnant females and growing fetuses to avoid metabolic disorders, which are associated with pregnancy.

8.3. Pregnancy diagnosis in goats

In caprine species, pregnancy recognition can be performed mainly by utilizing ultrasonography, and hormonal profiling, but sometimes they give false-positive results. ELISA can recognize PAG in serum/plasma of pregnant does at least 30 days after the breeding. Utilization of particular antisera permitted the segregation between pregnant and non-pregnant goats as early as 21 days after breeding. Its utilization in farm conditions is suggested after day 26 and day 32 in plasma and milk samples, respectively[49].

Temporal profiles of PAG concentration in whole and skim milk of pregnant and non-pregnant goats during early pregnancy were analyzed by Singh *et al*[46]. According to their study, PAG profiles in whole and skim milk of pregnant goats are characterized by relatively unchanged up to about day 24 post-mating followed by gradual increase up to day 30 and thereafter rapid increase from day 30 to day 51 of gestation, whereas the concentration of PAG in milk samples of non-pregnant goats remain relatively at the same level below cut-off values. Day 26 post-mating was identified as the first time point for significantly higher PAG concentration in skim and whole milk of pregnant goats than the non-pregnant goats[50].

As reported, in plasma and milk the circulating PAG level steadily increased as the pregnancy progresses and thus can effectively be used as a biomarker of pregnancy diagnosis during early gestation in goats. The good accuracy of the assay and strong positive relationship with a high agreement between milk and plasma PAG levels indicate suitability of milk samples for monitoring fetal well-being. Thus, it provides an alternative tool for traditional blood-based pregnancy detection tests and ultrasonography method in goats[50].

9. Conclusion and prospects

In conclusion, the best reproductive management in domestic animals is possible by precise pregnancy diagnosis which in turn depicts animals, fertility. PAG can also be used for accurate pregnancy diagnosis in farm animals. PAG, which is first discovered in ruminants, is a heterologous subgroup of aspartic proteases. PAGs are also expressed in other mammal species. They are synthesized by the external layer of trophoblast and then release in the maternal circulation. Therefore, the determination of the concentration of the PAG in maternal circulation is a useful tool for pregnancy diagnosis, for embryonic or fetal mortalities, and protein-protein interaction.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgements

The authors would like to express their gratitude to the ICAR- Central Institute for Research on Goats and GLA University for their support.

Authors' contributions

Nandini Sharma carried out the literature review and drafted the manuscript. Shiva Pratap Singh helped to draft the manuscript and gave suggestions. Alok Bharadwaj and Ramachandran Natesan gave the vision and suggestions regarding the importance of the study and helped to draft the manuscript.

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