

Popular Article

Pregnancy Associated Glycoprotein (PAGs) as an early pregnancy biomarker in ruminants - An update

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Introduction

Pregnancy diagnosis is an important part in the reproduction management of ruminants. In the last few years, a polymorphic family of placenta-expressed proteins has been discovered in ruminant species and used for pregnancy diagnosis. The pregnancy-associated glycoproteins (PAGs) are one of the biochemical compounds that signify early pregnancy and are synthesized in the mono- and binucleate cells of the ruminant's trophoblast. Part of them is released into maternal blood circulation, where they can be assayed by different RIA and ELISA systems. Due to the large variety of expressed molecules and the large variations in the post-translational processing of the glycoproteins, different immune systems present different abilities to quantify the PAG released in the blood. Recent investigations showed that, surprisingly, the level of milk production in ruminants can modify the concentration of PAG circulating in blood. On the whole, the data show that the RIA methods are very precise for measuring PAG concentrations in the maternal blood and milk of the ruminants. The sensitivity and specificity of this method are very high. The research results showed the possibility

of using PAG in milk and in blood as a pregnancy test. It is especially helpful in the diagnosis of gestation and in the detection of embryonic mortality as a non-stressed method in the pregnancy management of the ruminants.

Pregnancy associated glycoproteins (PAGs)

The foetal and maternal tissues are in close contact with each other by interdigitation of foetal villi into maternal crypts. There are two populations of trophoblastic cells: one is polarised mononucleated trophoblast cells, and another is trophoblast giant cells (TGC). TGC are mostly binucleated, nonpolarized, and migrate through the chorionic epithelium to fuse with uterine epithelial cells. The TGC evolve from mononucleated trophoblast cells by acytokinetic mitosis and are able to migrate from the foetus into the maternal compartment. The main function of TGC is the production and delivery of proteins and steroid hormones into the maternal compartment. TGC additionally contains a variety of signalling molecules, such as placental lactogen and pregnancy associated glycoprotein. Furthermore, several growth factor systems, such as vascular endothelial growth factor, platelet-activating factor, fibroblast growth

factor, and epidermal growth factor (EGF), are co-localised either in TGC or the uterine epithelium.

The PAGs consist of a large family of more than 20 closely related proteins that are only produced by the placenta. PAGs have been identified in domestic cattle, sheep, goats, zebu, water buffalo, and American and European bison. PAG, also known as pregnancy-specific protein B, constitutes a large family of glycoproteins, specifically expressed in the outer epithelial cell layer (chorion/trophoblast) of the placenta in several Eutherian species. PAG in maternal circulation was first described in 1982, when two proteins were isolated from the bovine placenta by immunoelectrophoresis: PSP-A and PSP-B. PSP-A, with a molecular weight (MW) of 65–70 kDa and an isoelectric point (pI) of 4.6–4.8, has been identified as α -fetoprotein that is not strictly limited to pregnancy; PSP-B was found to be a specific gestation protein with a MW between 47 and 53 kDa and a pI of 4.0 - 4.4. These PAG are members of the aspartic proteinase family, having high sequence homology with each other as well as with pepsin, pepsinogen, chymosin, cathepsin D and cathepsin E. The mature PAG polypeptide is approximately 330 amino acids long with a relative molecular weight (MW) of about 37 to 67 kDa. PAG can be detected in maternal blood soon after implantation and is used in pregnancy diagnosis and to detect the health of the foetus, placental abnormalities, embryonic/foetal mortality, and abortion. PAG concentrations in maternal blood have been used both for pregnancy diagnosis and as a marker of foetal/placental well-being or to monitor pregnancy failure during the late embryonic and early foetal periods.

a. PAGs in cows: In cows, PAGs have been detected in the maternal blood as early as days 15 to 22 or day 22 after conception. The PAGs detection results are more accurate from days 28 to 30 onwards. The levels of PAGs progressively increase in pregnant animals between the 6th and 35th weeks, and continue to rise more rapidly thereafter between the 35th and last weeks of gestation. Their concentrations then triple, more precisely between 20- and 10-days pre-partum, up to their maximum increase, which occurs about 5-10 days before calving. In cows, the concentration of PAGs at 28 to 30 days of pregnancy ranges from more than 0.5 to 0.8 ng/ml, with 0.8 ng/ml constituting the threshold for a positive pregnancy diagnosis. In early and mid-gestation, PAG concentrations increase slowly and gradually, remaining more than 160 ng/mL until day 240. Around parturition, concentrations increase with peaks of 1000 to 5000 ng/mL. This molecule kept secreted along the gestation period and remained until 4-5 days after parturition because of its long half-life. It has been observed that slow disappearance of PAGs from maternal blood after calving is due to the presence of high concentrations at calving, and also to the long half-life of bovine PAGs, estimated to be 7.5-9 days in European cows and around 9-10 days in African Azawak zebu cows. The original PAG (PSBP) test is commercially available as a trade name of "BioPRYN." The PAGs from the previous pregnancy are found within the bloodstream for several months after calving. It is necessary; therefore, to diagnose early pregnancy using PAG tests in the early stages of the postpartum period is at least 60 to 90 days postpartum before testing a cow for pregnancy when the standard BioPRYN® kit is used. The

second new commercially available test is being marketed by IDEXX laboratories (Westbrook, ME). The test can be used after 28 days of pregnancy, and results are available within 2.5 hours. Cows that are 60 days or more postpartum can be tested. The concentrations of PAGs in milk appear to be 10 to 50 times lower than those of the plasma, but their profile during the course of pregnancy is comparable. In the post-partum period, however, the decline in the PAGs' concentrations is faster in the milk than in the blood. It has been shown that, within 6 days of calving, the PAGs' concentrations decrease by 50% in plasma and by 95% in milk. Recently, using ELISA newly developed and reported that the amount of PAG found in milk was 1.5% of the amount present in serum. The milk test identifies pregnancy at day 40 post-insemination, with a threshold value of 0.0165 ng/mL. Low concentrations of PAG in milk samples is still a problem for pregnancy diagnosis.

b. PAGs in Buffaloes: The RIA-706 system, which uses antisera raised against caprine PAGs, was the first to be adopted for detecting PAG molecules in buffaloes, later RIA specific system for buffaloes (RIA860) used. With this system, pregnancy is detected in the time window from days 31-35 post mating. By using the same RIA system, observed great differences in the PAG concentrations between pregnant and non-pregnant buffaloes from day 28 onward. During pregnancy, the PAG concentration increased up to day 105 and then remained constant until parturition, different from bovine animals, for which, as described before, an exponential increase was observed until parturition. In the post-partum period, the PAG concentrations

decrease rapidly, reaching minimum values (<1 ng/mL) on day 30. Unlike cows, the rapid PAG disappearance in buffaloes does not require a cut-off limit in post-partum animals as a means for detecting a new pregnancy, as there is a voluntary waiting period of at least 50 days. The half-life, found to be 8.5 days, proved to be shorter in buffaloes than in cows.

c. PAGs in Ewe and Does: The pregnancy-related proteins identified in ewe were named SBU-3, PSPB, and PAG. Doe PAGs are glycoproteins with a MW ranging from 43 to 70 kDa and a pI ranging from 4 to 6.8. In ewes: plasma PAG concentration increases rapidly between 3 and 9 weeks of pregnancy. During the subsequent weeks the concentration decreases; then a marked increase is observed during the final weeks of pregnancy. Similarly, in does, there is a marked increase in plasma PAG concentration from around 3 weeks after breeding. A peak concentration is reached at 6–8 weeks of pregnancy, followed by a slightly decreasing trend during the remaining part of pregnancy. After parturition, a decline in PAG concentration within 3–4 weeks has been reported. But there are no studies describing the half-lives of PAG postpartum in these species.

Detected PAG in foetal serum of calves

Large individual variations were observed; however, concentrations were generally very low as compared with those measured in the dam, and tended to decrease with increasing foetal age. There is little information about how PAG are transferred from the mother to the offspring.

Early Pregnancy Factor (EPF)/Early Conception Factor (ECF)

EPF is an immunosuppressive glycoprotein associated with pregnancy. It was first identified in the mouse and subsequently in a large number of domestic species; in a cow it has a molecular weight of 200000. Commercially available test kits are available that use the dipstick principle and can detect early conception factor (ECF) in serum and milk from as early as 3 days after AI, although more accurate results are obtained if samples are taken at 7-8 days. However, in a more recent report, the accuracy of a commercial test still appeared to be unacceptably low. Obviously, an accurate early pregnancy test would have important practical application in the early identification of the non-pregnant cow in the luteal phase after an unsuccessful insemination; thus, the cow can be treated with $\text{PGF}_2\alpha$ to induce a premature oestrus, when it can be rebred, thus saving time.

Conclusion

The family of pregnancy-associated glycoproteins appears as a heterologous subgroup of the aspartic proteases. First discovered in ruminants, they are also expressed in other mammal's species. Their synthesis in the superficial layers of the trophoblast can be followed by their release in the maternal blood circulation. So, the use of the determination of their concentration can help for early pregnancy diagnosis and for new investigations on embryonic or foetal mortalities.