A review and update of research on pregnancy associated glycoproteins (PAGs) in cattle

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Key points

- PAGs are a large gene family found in all ruminants.
- The function that PAGs play during gestation remains a mystery. In this review speculation about putative roles for the PAGs will be highlighted.
- The PAGs display a wide range of expression patterns and localization. These proteins may be playing different roles depending on the exact location or time of pregnancy that they arise.
- Understanding the roles that PAGs play in ruminant pregnancy may lead to a better understanding of pregnancy success and failure.

Introduction

The placenta is a multifaceted organ that has a critical role in maintaining and protecting the developing fetus by transferring nutrients and metabolic wastes, acting as a regulator of the maternal immune system, and serving as a major endocrine organ. In the ruminant placenta there is a unique cell type (binucleate cells) that constitutes 15 - 20% of the fetal placental trophoblasts. These cells become visible around d 19-20 of gestation in cattle and have been shown to secret a plethora of hormones and proteins, including placental lactogen (PL) and pregnancy associated glycoproteins (PAGs). Although much of the focus on PAGs has been directed toward PAGs expressed in bincucleate cells, there are PAGs that have been shown to be expressed by mononucleated cells as well. This section will focus on the characterization and functions of PAGs in cattle and attempt to link data presented in the review of "Application of PAGs to manage reproductive efficiency in cattle."

Characterization of pregnancy associated glycoproteins (PAGs)

PAGs were first reported by Butler after isolation of two proteins from fetal membrane extracts, referred to at the time as pregnancy specific proteins A and B (PSPA/PSPB). In the same study it was determined that PSPA was alpha-fetoprotein and that PSPB was specific to the placenta. Sasser developed a PSPB specific radioimmunoassay which they used to measure PSPB in the maternal circulation. In subsequent reports, they demonstrated that measurement of PSPB in females could be used to successfully detect pregnancy in dairy cattle, sheep, and goats. Around the same time, Zoli reported the purification of another pregnancy specific protein that they called ‘bovine pregnancy associated glycoprotein’ (PAG). Bovine PAG and PSPB had very similar amino acid sequences at the amino-terminal suggesting that these two proteins were similar, if not identical.

Initial immunolocalization studies determined that PAG was synthesized by the trophoblast binucleate cells and stored in large secretory granules prior to delivery into the maternal circulation. The exact function of PAG remained unclear; however, Xie reported that 60% of the nucleotide sequence of PAG was shared with pepsinogens. Furthermore, it was shown that mutations in and around the active site rendered PAG inactive as a proteinase.

As time progressed, new members of the PAG family were discovered in cattle and many species within the Ruminantia suborder. Consequently, the original PAG was renamed PAG1 and PAGs discovered afterwards were numbered sequentially. It is now known that PAGs comprise a large diverse gene family belonging to the aspartic proteinase superfamily. In cattle alone, there are >20 distinct PAG cDNAs represented in Genbank.

Hughes reported that PAGs can be divided into two distinct groups: 1) ancient PAGs which are estimated to have originated about 83 million years ago (around the time the Artiodactyla order is thought to have arisen), and 2) modern PAGs which are estimated to have originated approximately 54 million years ago. These two distinct groups of PAGs have been studied extensively and characterized based on
their mRNA expression. Ancient PAG mRNAs are usually expressed throughout gestation in both mononucleate and binucleate trophoblast cells of the cotyledons; whereas, modern PAGs are synthesized primarily in binucleate cells of the trophoblast and their expression seems to change during gestation.13

Ancient PAGs

The ancient lineage of PAGs seems to have arisen from duplication of a single pepsinogen F-like gene around 85 million years ago.16 Kumar17 also reported the divergence of the even-toed ungulates (Artiodactyla) and odd toed ungulates (Perissodactyla) around this same time period suggesting that these two events may be closely related. In cattle, the ancient PAGs are comprised of a relatively small group of about six genes.13 These six bovine (b) PAGs are expressed in cotyledons from early placentation to term, in both uninucleate and binucleate trophoblast cells. Following secretion, some of the ancient PAGs accumulate at the microvillar junction between the maternal and fetal interface.18 Their function is still unknown, however, based on their localization. Wooding18 suggested that ancient PAGs may be important for the following: 1) adhesion of the uterus and trophoblast cells to maintain appropriate transport, 2) proteolytic processing, 3) activation of growth factors or bioactive molecules, or 4) protection of trophoblast cells from the maternal immune system. There is also speculation that the ancient PAGs and modern PAGs may work together throughout gestation. Collectively, the ancient PAGs were thought to be peptidases, although there had been no solid evidence until Telugu19 reported that bPAG2, which is the most abundant transcript reported in the PAG family possessed proteolytic activity. A similar PAG, bPAG12, has also been shown to be proteolytically active. The preceding authors concluded that ancient PAGs exhibiting proteolytic activity may function as sheddases to activate latent biomolecules, which could be important for placental development and growth.

Modern PAGs

The burst in gene duplication that led to the lineage of modern PAGs has been linked to the emergence of the synepitheliochorial placenta of the ruminant ungulates.16,18 The modern family of ruminant PAGs includes a larger number of genes than their ancient counterparts.13 Wooding18 suggested that the modern PAG family expansion could potentially have evolved to deliver a variety of fetal products and hormones to the mother by bypassing the uterine epithelial barrier. These PAGs are restricted to ruminant species and are expressed primarily in trophoblast binucleate cells from which they are released into the maternal system, with some accumulating in the stromal layer within the maternal caruncles.18 The authors concluded that the preceding localization pattern could potentially place PAGs in a position to engage in immunological protection, such as blocking lymphocyte or polymorphonuclear leukocyte migration and activation. To date there have been no clear functions related to modern PAGs; however, PAGs have been shown to inhibit different immune cells, in vitro, and may camouflage fetal/placental antigens from the immune system.20 Alternatively, PAGs have been suggested to have a luteotrophic action based on a report that addition of PSPB/PAG1 to endometrial cells increased the production of the luteal-promoting prostaglandin, prostaglandin E2 (PGE2);21,22 however, the evidence for a luteotrophic or antiluteolytic action of PAGs is not compelling at this time.

Possible potential function(s) of the PAG

To date the function of PAGs is not clear. However, their expression patterns by the placenta of cattle and related species as well as the proteolytic activity of some PAGs could provide some insight into their roles during pregnancy. For instance; many PAGs such as bPAG-2 and porcine PAG-2 (belong to the ancient PAG group) are found to accumulate at the placental feto-maternal interface. Bovine PAG-2 and procine PAG-2 are known to have a proteolytic activity,19,23 which is suggestive of possible roles involving protein turnover or remodeling at the trophoblast-uterine epithelial interface18 or they could be acting to proteolytically activate bioactive molecules and latent growth factors located at the interface.24,25 There are some PAGs that lack the ability to act as proteinases; these may have another role, such as peptide binding at the uterine-fetal interface. Interestingly, PAGs are able to interact with peptide
ligands via their substrate-binding cleft. Conceivably, binding to other proteins through the substrate-binding cleft could position PAGs to interact with other proteins at the maternal-fetal interface or with transmembrane receptors (e.g., integrins). Furthermore, the carbohydrates displayed on the surface of PAGs could bind to a lectin (carbohydrate-binding protein) at the maternal-fetal interface to sequester them to that location. Pregnancy associated glycoproteins with enzymatic activity typically exhibit proteolysis of substrates at comparatively low pH. The positioning of proteinases at the interface could facilitate release of the cotyledon from the caruncular crypts around parturition when the pH of the interface microenvironment falls and proteolytic activity of these PAGs would be expected to increase.

Pregnancy associated glycoproteins may have an effect on the maternal immunological system in cattle. For example, bPAG-1/PSPB treatment of bovine bone marrow has been shown to cause a drop in bovine hematopoietic cells proliferation. In other experiments, bPAG-1/PSPB treatment of bovine endometrial (BEND) cells induced release of granulocyte chemotactic protein 2 (GCP2). Pregnancy associated glycoproteins have also been shown to associate with the peripheral blood lymphocytes and with endometrial serpin-like proteins in vitro.

In cattle and sheep PAG1/PSPB could have an effect on luteolytic activity. For instance, bovine luteal cells progesterone and prostaglandin E2 (PGE2) production increased in response to PSPB treatment. This observation of an increase in bovine luteal cells progesterone production might be due to a luteotropic effect of PGE2 but it was not observed consistently. It seems to be that PAGs can play multiple roles during pregnancy in regard to placental development and function.

In addition, PAGs may be playing a totally different role than any of the data above suggest. Recent data generated out of Dr. Jon Green's lab at the University of Missouri is potentially pointing to a role of PAGs and uterine remodeling around the time of early placental attachment. These preliminary data found that the addition of purified bovine PAGs to endometrial explants, collected on day 18 from pregnant and nonpregnant heifers increased the expression of members of the matrix metalloproteinase gene family which have a role in tissue remodeling. Furthermore, we have demonstrated that cows likely to undergo late embryonic mortality (between day 28-60 of gestation) have decreased circulating concentrations of PAGs on day 28 compared to cows that will maintain a pregnancy. These results support the idea that cattle pregnant on day 28 of gestation, with decreased circulating concentrations of PAGs, may be undergoing pregnancy loss based on the failure of placental and endometrial crosstalk along with a failure of tissue remodeling that is critical for early placentome formation.

Summary

The bovine PAG family is a large group of related proteins that are encoded by more than 20 genes. The function that PAGs play during gestation have not been elucidated, but preliminary data point to having a role in manipulation of the maternal immunological system, regulation of luteolytic activity, and(or) tissue remodeling. As our understanding of mammalian genomes increases and the advancement of biological tools increase it is likely that the exact function that PAGs play during pregnancy will be discovered.

References