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REVIEW

Biology of the Placental Proteins in Domestic Ruminants: Expression, Proposed Roles and Practical Applications

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Abstract

The placenta, a lodgment at the feto-maternal interface, is a temporal organ that plays crucial roles in maintaining gestation and fetal development. To achieve these purposes, the placenta produces an array of proteins with distinct spatio-temporal characteristics. In this review, the authors focus on the paralog families of prolactin (PRL) and aspartic proteinase, exclusively produced by the placenta, and discuss their biological roles and practical significance in animal husbandry. The bovine placental PRL family consists of one classical member and at least ten non-classical members. Bovine placental lactogen (PL) is a unique classical member due to its lactogenic activity and potentially involved in partitioning nutrients to maintain fetal development. In contrast, the biological roles of non-classical members in bovine placental PRL family proteins remain unclear. Recent papers have reported that a non-classical member protein named prolactin-related protein -I (PRP-I) exhibited angiogenic activity following C-terminal cleavage by proteolytic enzymes. These results suggest that non-classical members of the placental PRL family exert their biological activities via specific mechanisms other than the PRL receptor-signaling pathway. The bovine genome contains a hundred or more aspartic proteinase-like genes and at least 22 mRNAs with close structural relationships are transcribed in the placenta. These molecules correspond to pregnancy-specific protein (PSP)-B, PSP-60 and pregnancy-associated glycoprotein (PAG) as reported previously. The true character of PSPs and PAG is thought to be a mixture of placental aspartic proteinase-like proteins. Immunological detection of PAG protein in maternal serum is the basis for early pregnancy diagnosis in ruminants. Currently, early pregnancy diagnosis by PAG assay is available for cows, buffaloes and deer.

Discipline: Animal industry
Additional key words: buffalo, cow, deer

Introduction

The female of the viviparous species gives birth to offspring capable of surviving outside the dam following a distinct gestation period. Except for monotremata, most mammals are viviparous species. Mammalian embryos dramatically develop throughout gestation in the maternal uterus and in cows in particular, life begins at fertilization at 0.2 mm in diameter, and thereafter the fetal instantaneous growth rate peaks at 350 g/day around day 220 of gestation and the fetal body weight reaches more than 35 kg at birth[43]. The fetus requires large quantities of oxygen and nutrients that cannot be sufficiently supplied by simple diffusion from the maternal endometrium. To maintain the massive fetal growth, the pregnant female forms a placenta as a lodgment for logistics between the dam and fetus. Maternal supplies come via the maternal blood, are absorbed by the placenta and then carried by the fetal blood to reach the target tissues.

The gross appearance of the placenta or afterbirth varies considerably among species, such as in ruminants where the afterbirth has multiple cotyledons on the chorion, whereas other domestic species like the mare and sow form a diffused placenta consisting of a simple and diffused apposition between the fetal trophoblast and the maternal endometrium. Ruminant cotyledonary villi interdigitate to specialized projections of the caruncular tissues

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to form a placentome as the basal unit of the placenta. A cotyledonary placenta is peculiar to ruminants.

In this review, the authors survey the morphological characteristics of trophoblast differentiation and placentogenesis in ruminants and then discuss the biological and practical aspects of ruminant placental proteins.

Trophoblast differentiation and placentogenesis in ruminants

Bovine trophoblast differentiation initiates at the late stage of morula[33]. In the early (non-compacted) morula stage, each cell between the embryonic and extra-embryonic lineages is indistinguishable. Around day 7 following fertilization, a trophectoderm layer develops on the outer side of a spherical blastocyst that is surrounded by the zona pellucida. After hatching from the zona pellucida, the trophectoderm layer further differentiates to form trophoblasts. By day 14 of pregnancy, a hatched embryo develops from a spherical to an ovoid shape, finally growing to 20 cm in longitudinal length around day 18[55]. By day 21 of gestation, trophoblasts closely appose to endometrial epithelium and binuclear trophoblasts (BNCs) are formed via the endoreduplication of mononuclear trophoblasts (MNCs) concomitant with the commencement of placental PRL family protein expression[78]. By day 30, considerable numbers of BNCs have developed in the trophoblast layer, with the trophoblastic and endometrial layers becoming attached in the locus of cotyledon that will be formed thereafter. The trophoblasts then form placental villi that interdigitate with the uterine epithelium to form the placenta. In ruminants, placentogenesis is restricted to the chorionic layers apposing to the uterine caruncle, but many placentae (up to 100) are formed corresponding with each caruncle[74].

Trophoblast cells play two important roles in placentogenesis and both secretion and migration are major characteristics of trophoblast lineages[24]. Trophoblasts produce many kinds of proteins on a massive scale, including hormones and proteolytic enzymes, and exhibit migratory behavior to remodel the feto-maternal interface and form the placenta[23]. Unlike in rodents and primates, ruminant trophoblasts do not intrude beyond the basal membrane of the maternal endometrium[74]. Ungulate species form semiplacenta, since their trophoblasts never intrude beyond the uterine basement membrane.

During placentogenesis, the BNC migrates toward the epithelium and fuses with a uterine epithelial cell to form a trinucleate cell (TNC). The cytological dynamics of TNC formation differ significantly from those of BNCs, since BNCs are formed via endoreduplication of a single MNC. Following TNC formation, secretory granules of BNC are released into maternal circulation and the materno-fetal hybrid TNCs further fuse with adjacent cells, resulting in the formation of a multicellular syncytium.

The trophoblast produces massive amounts of proteins during gestation. In many mammalian species, the placenta produces pituitary hormones or a member of the corresponding paralog family. Human (horse) placentae produce human (equine) chorionic gonadotropin (hCG or eCG). Like other pituitary gonadotropins, hCG consists of two subunits. The alpha subunit of hCG is identical to the consensus gonadotropin alpha subunit expressed in the pituitary, while the beta subunit is a paralog of the luteinizing hormone (LH) beta subunit. Additionally, eCG has amino-acid sequences identical to those of equine LH. Gonadotropin alpha and LH beta genes are transcribed in both the pituitary and placenta to generate LH and eCG, although the carbohydrate modifications differ considerably between LH and eCG.

Placental prolactin family proteins

The placenta produces an array of proteins structurally and functionally similar to pituitary PRL. Hormones that are paralogous with PRL are known as the placental PRL family. In many species, the placenta expresses PRL (in primates) and its paralogs (in primates, rodents and ruminants).

1. History

Placental PRL family proteins were first identified as a mammotropic action of placental tissue when co-organ-cultured with mammary gland explants[30]. The placental factor that stimulates lactogenesis was named placental lactogen (PL). However, in some gene databases (e.g. GenBank, http://www.ncbi.nlm.nih.gov/genbank/), the term chorionic somatomammotropin (CSH) is also used to describe PL, since PL from some species exhibit somatogenic activity. The presence of bovine PL was first reported in the mid-1970s via a co-organ-culture strategy with placenta and mammary tissues[10].

2. Gene family, expression, protein structure and biochemical characteristics

To date, PL has been reported in many mammals, though not in rabbits and dogs[60]. In rodents and ruminants, placental PRL family genes have also been expanded by gene duplication[69]. Over 20 (10) genes of the PRL family are expressed in rodent (ruminant) placenta. Recent bovine genome analysis has revealed that PRL, PL, prolactin-related protein (PRP) and growth hormone (GH) are co-localized on chromosome 23 (http://genomes.arc.georgetown.edu/bovine/genepages/genes/BT12480),[15].

These genes are expressed with distinct temporo-spatial patterns[65, 66, 71].

The complementary DNA sequence encoding bPRL (GenBank; NM_173953) contains a 907-nucleotide (nt) open reading frame and a 690-nt coding region for preprolactin. The deduced amino acid sequence of bPRL contains 30a.a. signal peptide and 199a.a. mature protein. In placental PRL family proteins (PL/PRP family), the signal peptide with 36a.a. is well conserved among the molecules. Although some PRPs contain signal peptides of different sizes (68a.a. in PRP II), the amino acid sequences of mature proteins are 199 to 200a.a. in length. Additionally, the positions of cysteine residues are highly conserved among individual members[64].

Since PRL is secreted as a polypeptide without glycosylation, the molecular weight of bPRL is 23kDa. However, all bovine placental PRL family proteins reported to date are glycoproteins with heavier molecular sizes exceeding 30kDa[57, 64]. Similar to hCG and eCG, there seem some beneficial aspects of placental glycoproteins in terms of increased bioavailability during gestation. Carbohydrate chains appear to delay the biological clearance of proteins to maintain sufficient concentration in the target tissue. The bPRL amino acid sequence has 51% (40 to 48%) identities with those of bPL (bPRPs)[64].

The biological signals of PRL are introduced into target cells via cell-surface receptors. Although lactogenic activity is one of the principal biological characteristics of PRL, only the PL is capable of stimulating the proliferation of Nb2 lymphoma cells in bovine placental PRL family proteins[64]. An Nb2 lymphoma cell bioassay is suitable for assaying the biological activity of PRL family proteins[51, 58]. The lactogenic activity of bPL has been reported as almost equipotent to that of bPRL[51]. In ruminant placental PRL family proteins, it is generally thought that members other than PL are unable to stimulate the proliferation of Nb2 cells. Therefore, based on lactogenic activity, bPL (other members) called "classical" ("non-classical") members.

Interestingly, ovine PL binds with high affinity to primate, rodent and bovine GH receptors[13, 38, 68]. However, the somatogenic activity of bPL is lower than that of GH. Although ruminant PLs are able to bind to GH receptors with higher affinity than GH, PL cannot activate GH receptor-mediated biological actions or act as antagonists. Similar findings have been reported regarding the interaction between the PL and PRL receptors. Since excessive concentrations of PL bind to PRL receptors at a ratio of 1:1, an occupied PRL receptor cannot actively induce PRL activities and these ligand-saturated receptors are unable to dimerize to mediate hormonal signals into the cell. In non-classical members of ruminant PRL family proteins, none of the target cells, receptors, or signal transduction pathways has yet been reported.

3. Biological roles

The biological roles of placental PRL family proteins have been extensively studied in rodent species[2, 52]. In rodent species, PRL and PL play critical roles in maintaining the corpus luteum during gestation. Twice-daily (diurnal and nocturnal) surges of pituitary PRL act as principal luteotropic signals to form and activate the corpus luteum during early pregnancy. After the implantation of embryos, twice-daily PRL surges are inhibited by PL-I produced by the trophoblast, and PRL secretion remains at a nadir until shortly before parturition[63]. Trophoblast-derived PL-I takes over from pituitary PRL in mid-pregnancy, while PL-II also takes over from PL-I in late pregnancy. PL-I and PL-II bind to the PRL receptor and operate as ligands of the PRL signaling pathway. The physiological roles of the rodent PL in maintaining the corpus luteum, fetal development, mammogenesis and maternal adaptation in pregnancy have been investigated in many studies[53].

Information about the biological roles of non-classical members in rodent species remains limited. Prolactin-like protein -A (PLP-A), PLP-E, proliferin (PLF) and PLF-related protein have been studied to determine their biological roles in pregnancy[1, 8, 29].

It has been suggested that ruminant PL is a factor in the partitioning of nutrients to maintain fetal development. An intriguing study has been reported about the relationship between the maternal nutritional status and fetal development[44]. In thin cows, although the uterine weights were less, both the chorioallantoic and cotyledonary weights exceeded those in moderate cows, although there was no significant difference in fetal growth between the two groups. Plasma PL concentrations were greater in thin cows than in moderate cows and the total fructose concentration in allantoic fluid was reduced in thin cows. Since allantoic fructose is a major energy source in the placenta, the greater allantoic and cotyledonary tissues seem one of the compensatory responses used to increase the amount of nutrients reaching the fetal compartment in thin cows. These results suggest that PL acts as a compensatory regulator to correct the debased nutritional status.

In contrast, the active immunization of oPL prior to conception in the ewe results in increased PL production in the placenta, a heavier birth weight in newborns and increased milk production following parturition[34]. In immunized ewes, PL could be immediately neutralized by its own antibody, but local PL production might be activated, boosting the bioavailability of PL in the feto-maternal compartment. Additionally, when anti-oPL autoantibody binds to its own PL, the antibody might protect circulating oPL from degradation, extend its half-life, and increase

the availability of oPL in mammary tissue.

In ruminants, the biological roles of non-classical members remain unclear and only limited information about the biochemical and biological characteristics of bPRP-I has been obtained. bPRP-I is transcribed in trophoblast binucleate cells[78] and secreted to the uterine lumen[32]. The secreted bPRP-I is deposited into the interstitial tissues of the utero-placental organ by anchoring with type IV collagen[59]. The affinity of placental PRL family proteins for extracellular matrices has also been reported in rodent d/tPRP that interacts with heparin proteoglycan[45, 46]. Capturing bPRP-I and d/tPRP by extracellular matrices might permit an increase in the local concentration of the ligand, restrict their biological actions near the secreted source, and control the timing of the release from the anchorage along with tissue remodeling during placentogenesis.

Recently, a physiological role of bPRP-I has been proposed[67]. Bovine PRP-I could be cleaved at the carboxyl-terminal region by proteolytic enzymes, with the resulting amino-terminal polypeptide acquiring a new biological activity quite distinct from parental protein: namely stimulating the proliferation of vascular endothelial cells. This finding implies that the N-terminal polypeptide of bPRP-I stimulates angiogenesis in the bovine placenta, which seems plausible because ovine PRP-I has a short polypeptide chain, comparable with N-terminal regions of bPRP-I and caprine PRP-I. The transcript encoding oPRP-I lacks a C-terminal domain corresponding to bovine and caprine PRP-Is, and the shifted reading frame results in premature termination. Therefore, approximately two-thirds of the polypeptide chain is translated. In terms of molecular weight, the size of the cleaved bPRP-I coincides well with that of oPRP-I. Although the biological activity of oPRP-I remains unknown, it is likely that peptides derived from bPRP-I have certain effects on placental angiogenesis in the cow.

Interestingly, rat PRL can be cleaved by cathepsin D between amino acid residues Y145 and L146, and between W148 and S149, resulting in 16k and 6k fragments and a tripeptide (L-V-W)[4, 5]. Among these peptides, the 16k fragment acquires novel bioactivity that is distinct from that of intact PRL: the 16k fragment inhibits endothelial cell proliferation, stimulates endothelial apoptosis, and inhibits angiogenesis[56]. Enzymatic cleavage of PRL by cathepsin D is also reported in humans as a causative molecule of postpartum cardiomyopathy[26]. The biochemical mechanisms in the opposing activities of N-terminal fragments between rat and human PRLs and bPRP-I are quite unknown. Rodent PLF stimulates angiogenesis but PLF-related protein inhibits it, although both proteins share certain amino acid residues[7, 29]. It is likely that the biological activity of PRL family protein varies considerably among the paralogs and orthologs.

4. Application for animal production

Bovine PL exerts mitogenic activity in mammary tissue, whereas bPRL does not, although bPRL is a more potent inducer of milk protein synthesis in vitro[12]. Since GH has reportedly boosted the increment of milk yield in cows[18, 42], whether or not the exogenous administration of recombinant bPL may increase milk yield is an intriguing question. The milk yield of dairy cows and heifers treated with recombinant bPL exceeds that of an untreated control, but recombinant GH stimulates a greater increase in the milk yield than bPL[11]. Therefore, in the United States, recombinant bGH (bovine somatotropin; bST) that has been modified for sustained release after administration is currently used to stimulate milk yield in commercial dairy farms[6]. The exogenous administration of GH increases plasma concentrations of non-esterified fatty acids (NEFAs) and glucose by inducing insulin resistance. In contrast, bPL has little or no effect on the increment of NEFAs. Dry matter intake (DMI) is increased by bPL but not by bGH. Therefore it is suggested that bPL is a potential factor for increasing milk production in vivo, by simply increasing DMI without altering insulin sensitivity and fatty acid mobilization.

5. Conclusion

The ruminant placenta produces an array of proteins that are structurally and functionally similar to pituitary PRL. Placental PRL family proteins are secreted from trophoblasts as glycoproteins. These molecules are classified as “classical members,” including PL capable of inducing lactogenesis, and “non-classical members,” including PRPs that cannot stimulate lactogenesis. The physiological roles of ruminant PRL family proteins remain largely unclear. Bovine PL has been supposed to be a partitioning factor in the allocation of nutrition between the dam and fetus. Recent studies have revealed that bPRP-I is anchored by type IV collagen and the enzymatic cleavage of bPRP-I by cathepsins or matrix metalloproteinases generates an N-terminal bPRP-I fragment that exerts angiogenic activity. The chronic administration of bPL to the cow stimulates the increment of milk yield, but the bPL potency is less than that of bGH.

Placental aspartic proteinase family proteins

The ability to determine whether or not an animal is pregnant is of value for farm economics. The procedure for early pregnancy diagnosis is also a key technology for improving reproductive performance in domestic species.

To minimize the calving interval, farm managers always want to know the service result as soon as possible. Many clinical and laboratory methods have been proposed, and several methods are now available to diagnose pregnancy. Evaluation of a pregnancy diagnosis procedure is based on the diagnosable stage, expense, accuracy and simplicity. Ultrasonography techniques are now widely used as the gold standard for early pregnancy diagnosis in bovines. An accurate diagnosis can be achieved by ultrasonography after 30 days of gestation in the cow.

For earlier diagnosis, some laboratory procedures that rely on the detection of pregnancy-specific changes in maternal body fluids, have been proposed. These methods are based on the immunological reaction detecting hormones, the early pregnancy factor and pregnancy-related proteins.

Previously, radioimmunoassay and enzyme-linked immunosorbent assays for measuring plasma or milk progesterone (P) concentrations were established for early pregnancy diagnosis in cows[37, 49]. Plasma or milk P concentrations in cows bearing an embryo tend to be consistently high around day 21, whereas in non-pregnant cows, the concentration declines prior to the return of estrus[25]. However, the assaying for plasma P concentration is not always suitable for pregnancy diagnosis, because the duration of the estrous cycle may vary considerably among individual cows[35].

For more accurate pregnancy diagnosis, some immunological methods were attempted to detect the factor derived from fetal compartment. The presence of bovine early pregnancy factor (EPF) or early conception factor (ECF) that was detected by a rosette-inhibition test has been reported[36]. Although the detection of EPF/ECF is potentially applicable as a procedure for bovine early pregnancy diagnosis, many lines of current research have concluded that the EPF/ECF test is unreliable for early pregnancy diagnosis in cows[3, 14, 19].

Consequently, searching immunoassay targets for pregnancy diagnosis resulted in the identification of novel molecules that are currently known as pregnancy-specific protein-B (PSP-B) or a pregnancy-associated glycoprotein (PAG).

1. History

Some immunological methods allowing even earlier pregnancy diagnosis have been studied. Immunological techniques rely on detecting antigen originating from the conceptus. Several proteins have been reported as "pregnancy-specific proteins" or "PAGs" In the 1980s, pioneering work identified two pregnancy-specific proteins in maternal peripheral serum in the cow, namely pregnancy-specific proteins (PSP)-A and –B[9] . PSP-A was bovine α-fetoprotein produced by the fetal yolk sac and liver. PSP-B was a novel protein without any immunological cross-reactivity with proteins known in those days. Since then, PSP-60 and pregnancy-associated glycoprotein have been reported independently[48, 77, 79]. It is now known that PSP-B, PSP-60 and PAG are secreted by placental trophoblasts and that these proteins are identical or very closely related[75].

2. Protein structure, biochemical characteristics, gene family and expression patterns

Biochemical and nucleotide sequencing analyses have revealed that ruminant PAGs are members of the aspartic proteinase family, with more than 50% amino acid homology to pepsin and, cathepsin-D and -E. Ruminant PAG protein has a cleft capable of binding to the substrates of aspartic proteinases. This characteristic is the basis for affinity purification of PAG proteins from the placenta using pepstatin A-coupled affinity resin[41, 54]. However, because of amino acid substitutions at potentially active sites of the molecule, ruminant PAG had been suggested as enzymatically inactive[77].

Since that time, the diversification of PAG genes has been reported[76]. Surprisingly, each ruminant species possesses 100 or more PAG genes in the genome, and most PAG genes are supposed to be exclusively transcribed in the placental trophoblasts. To date, at least 22 mRNAs encoding the bPAG family have been reported. The nucleotide sequences were highly conserved among these transcripts. Subsequent studies revealed that PSP/PAG represents a mixture of related proteins. Close relationships among the PAG, PSP-60 and PSP-B proteins are convincing, owing to both the highly conserved molecular family and the cross-reactivity of polyclonal antibodies to related molecules.

PAG genes are classified into two subfamilies, ancient and modern, based on their molecular evolution[22, 28]. The ancient PAGs appear to possess some or all of the characteristics of an ancestral molecule, specifically an enzymatically active aspartic proteinase, and are expressed in trophoblast MNCs and BNCs. The ancient PAG subfamily contains PAG-2, -8, -10, -11 and -12[62]. Conversely, modern PAGs appear to be enzymatically inactive as an aspartic proteinase and are exclusively expressed in trophoblast BNCs. It is presumed that modern PAG genes originated 52±6 million years ago, then rapidly evolved by gene duplication after the divergence of ruminant species[28]. The spatial pattern of PAG expression, whether in binucleates alone or both mononucleates and binucleates, is subject to their phylogenetic relationships. Additionally, PAGs are also expressed in temporally distinct patterns during gestation in both bovine and ovine species. In cows, the ancient

PAG members are expressed throughout gestation, however the expressions of modern PAG molecules appear to be restricted within a specific stage of gestation[22].

3. Biological roles

Since most PAGs are supposed to be inactive aspartic proteinase-like proteins, their biological roles remain unclear. Those placenta-derived proteins that rapidly evolved by simple gene duplication might be just oddities without any specific biological significance. However, in view of their evolutional survival and distinct expression patterns, the plethora of PAG paralogs implies that they play crucial roles during gestation.

The immunosuppressive action of PAG has been reported by Belgian scientists. They reported that PAG inhibited the proliferation of bone marrow progenitor cells *in vitro* and concluded that PAG might contribute to increased susceptibility to perinatal mastitis in dairy cows[16, 27]. However, during the perinatal period, immunological circumstances change dramatically according to the physiological conditions. Further research is required to justify extending this deduction from the results of an *in vitro* culture experiment to whole-body pathology.

Recent papers have reported that some ancient PAGs exhibit proteolytic activities as aspartic proteinase[61, 62]. These ancient PAGs might act as a secreted aspartic proteinase during gestation, while cathepsins are localized in the cell.

4. Application for animal reproduction

PSP/PAG was initially identified during a search for placenta-specific antigens as a target of immunological pregnancy diagnosis. Subsequently, a radioimmunoassay for PSP-B in bovine peripheral serum was established in the mid-1980s[48]. The serum concentration of PSP-B in pregnant cows could be detected 24 days after breeding. The PSP-B measurement could detect pregnancy earlier and more simply and accurately than conventional rectal palpation. Serum bPAG concentration is first detectable on day 24 post-breeding, whereupon it increases steadily as gestation progresses and dramatically increases in the prepartum period. The serum bPAG concentration peaks at the time of parturition at 1000ng/ml or more[40, 48, 80]. In twin pregnancies, maternal serum PAG concentrations exceed those in a singleton, probably owing to the robust placental mass in the twin[39].

PAG concentrations in maternal sera seem considerably higher than those of other peptide hormones, which might result from a higher production rate and/or slow clearance of the protein due to its long half-life in peripheral circulation. Although the instantaneous production rate of PAG is unknown, the decrement profile of serum PAG concentration following parturition suggests that slow clearance is a key factor for maintaining peripheral concentrations during gestation[80]. After calving, the slow clearance rate of PAG in maternal circulation could result in the continued presence of remnant PAG protein nearly 100 days after parturition. This presents a possible problem for early pregnancy diagnosis by serum PAG measurement when re-breeding occurs within 100 days postpartum[80].

To solve this problem, Green and co-workers focused on the temporal pattern of each member gene of the PAG family, since they previously published distinct temporal profiles of PAG family members[20, 21]. The sandwich ELISA system, which uses both monoclonal and polyclonal antibodies targeting early-pregnancy PAGs, has been established and is currently available in the market. However, it has been reported that in rare cases ELISA detected immunoreactive PAG at day 15 post-breeding[20]. The cause of peculiar results, possibly due to an accessory source of immunoreactive PAG, is unknown.

Early pregnancy diagnosis via serum PAG measurement could also apply to other ruminants, especially deer and buffaloes. In elk[70], fallow deer[72, 73] and reindeer[47, 50], serum PAG detection provides a useful tool to diagnose pregnancy in animals. Indeed, some trials for pregnancy diagnosis in buffaloes by serum PAG assay have been reported[17, 31].

5. Conclusion

The ruminant genome contains one hundred or more genes that are structurally similar to aspartic proteinases. Among these, at least 22 mRNAs are transcribed in the placenta. Placenta-derived aspartic proteinase-like proteins are called PAGs. Placenta-derived PAGs are classified into two categories, the ancient and modern subfamilies. The ancient PAGs appear to possess active aspartic proteinase activity and are expressed in trophoblast MNCs and BNCs. Conversely, modern PAGs have lost all enzymatic activity and are exclusively expressed in trophoblast BNCs. The biological roles of ancient and modern PAGs remain almost unclear. Immunological detection of PAG in maternal peripheral serum can indicate pregnancy in cows earlier than the conventional procedure by rectal palpation. Early pregnancy diagnosis by maternal serum PAG detection could be applied to other ruminants.

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