Journal of
Advanced Veterinary Research
Volume 2 (2012) 50-58 **Review Article**

An overview on Pregnancy Associated Glycoproteins in Cattle and Buffalo

Jerome A.*

Division of Animal Reproduction, Indian Veterinary Research Institute,Uttar Pradesh, India-243122.

(Recieved 14 October 2011/ Accepted 18 December 2011)

Abstract

Accurate pregnancy detection is one of the prime requisites in reproductive management of domestic animals as it indirectly depicts fertility of the animal. Early pregnancy detection in animals is important so as to monitor reproductive losses. Though hormonal profiling of progesterone is an important method of pregnancy detection in domestic species; it usually results in false positivity. Pregnancy marker(s), which is embryo specific and depicts presence and viability of the embryo, should be considered ideal candidate for developing pregnancy diagnostics. Pregnant Associated Glycoproteins (PAGs) are potential biomarker in early pregnancy in cattle and buffalo. They belong to the aspartic proteinase family but proteolytically inactive due to key mutations. In this review, the scope and prospects of pregnancy associated glycoproteins as potential pregnancy biomarkers in cattle and buffalo species, is discussed.

Keywords: Conceptus; Biomarkers; Pregnancy diagnosis; Pregnancy Associated Glycoprotein

Introduction

Optimum reproductive efficiency in domestic species is obtained by better conception rate which in turn is affected by the series of successful interactions between the conceptus and maternal reproductive tract during pregnancy. These interactions between the conceptus and maternal system are important both for maternal recognition of pregnancy as well as embryonic development by blocking PGF2α secretion from the uterus, sustaining the developing conceptus (Spencer *et al*., 2004). Therefore, establishment of pregnancy involves synchronous interactions between two interdependent systems i.e. the conceptus (embryo and extra embryonic membranes) and the maternal system. The synchrony between these systems during the peri-attachment period is influenced by potential signals from the conceptus which facilitate in recognition of pregnancy.

These signals from conceptus to the maternal unit are crucial because they interrupt normal cyclic luteal regression thereby enhancing embryonic survival and sustenance of pregnancy. The important signals to maternal system to sustain pregnancy are mainly proteins or steroids (Sousa *et al*., 2006, Szafranska *et al*., 2006, Roberts *et al*., 2007). These signals can be considered as potential markers of embryonic viability as they influence pregnancy recognition and successful implantation. Pregnancy associated glycoprotein is one such bimolecular secreted during pregnancy and possess functions viz. embryo protective, placentogenesis, placental remodeling and maternal recognition of pregnancy and sustenance of pregnancy. This review throws some light on pregnancy associated glycoprotein, its genomic characteristics and physiological functions in cattle and buffaloes.

Pregnant Associated Glycoproteins (PAGs)

Pregnant associated glycoproteins comprise a large group of placental Aspartic Proteinase superfamily that is expressed in pre-placental trophoblast (TR) and after implantation in the trophoectoderm (TRD) – the chorionic epithelium of eutherian mammals with different placenta types. The first member of the family was isolated by Butler *et al*. (1982) from bovine placenta and named as pregnancy specific protein B (PSPB). Subsequently, re-

*Corresponding author: Jerome A
Address: Division of Animal Reproduction, Indian Veterinary Research Institute,Uttar Pradesh, India-243122.
E-mail address:: jerome210982@gmail.com

Table 1. Native PAG protein isolated from placental tissues of different species

Name	Molecular weight (kDa)	pH	Day of pregnancy	Species	Reference
bubPAG	52-77	-	Mid-late pregnancy	Buffalo	Barbato *et al.* (2008), Singh *et al.* (2005)
bPAG-1	67	4.4-5.4	90	Cattle	Xie *et al.* (1991), Zoli *et al.* (1991), Butler *et al.* (1982)
zebuPAG-1	51-69	4.4-6.7	70	Zebu cattle	Sousa *et al.* (2002)
oPAG-1	47-90	4.1-5.9	15-25	Sheep	Willard *et al.* (1995)
cPAG-1	62,59,55	4.9-6.2	48-69	Goat	Garbayo *et al.* (1998)
pPAG-1	42-79	-	15-77	Pig	Szafranska *et al.* (1995)
ePAG	37,41	4.8-6.2	15	Horse	Green *et al.* (1999)
EbPAG	50-71	3.7-7.4	45-120	European bison	Kiewisz *et al.* (2008)
AmPAG	56-75	4-4.6	120	American bison	Kiewisz *et al.* (2008)

lated proteins have been identified with different names such as PAG-1 (Xie *et al*., 1991, Zoli *et al*., 1991), PSP-60 (Mialon *et al*., 1993), SBU3 (Atkinson *et al*., 1993) in ruminant ungulates. These proteins are detectable in maternal blood and are reported to be useful as prenatal markers for pregnancy diagnosis in various domestic and wild ruminants. PAGs were isolated using biochemical procedures from cotyledons in cattle (Zoli *et al*., 1991, Sousa *et al*., 2002, Klisch *et al*., 2005), ewe (Xie *et al*., 1997, El Amiri *et al*., 2003, 2004), goat (Garbayo *et al*., 1998), buffalo (Barbato *et al*., 2003, 2008; Singh *et al.*, 2005) and bison (Kiewisz *et al.*, 2008). Purified and semi-purified preparations were used to immunized rabbits and the antisera has been used to develop homologous (Sasser *et al*., 1986; Humblot *et al*., 1988; Zoli *et al*., 1992; Mialon *et al*.,1993,1994, El Amiri *et al.*, 2006, 2007) and heterologous radioimmunoassay (RIA) systems (Ranilla *et al*., 1994, Gonzalez *et al*., 1999, Ayad *et al*., 2007) as well as enzyme linked immunosorbent assay (ELISA) systems (Green *et al*., 2005). Apparent molecular mass of purified PAG in cattle ranges between 37 to 78 kDa. The variable degree of glycosylation in different PAG plays important role in regulating the half life of the PAG proteins. There are various PAGs (PAG-1 to PAG-22) secreted from placenta at different stages of pregnancy. The first PAG purified from bovine placenta is known as boPAG-1 (Xie *et al*., 1991, Zoli *et al*., 1991). It has molecular weight of 67 kDa and has various isoforms (pI 4.4, 4.6, 5.2 and 5.4). In bovine, PAG-1, -3, -4, -5, -6,-7 and 9 are expressed in binucleate cells (BNC), while boPAG-2, -8, -10, -13 are expressed in both mononucleate cells (MNC) and binucleate cells (Green *et al*., 2000). Other PAGs isolated from bovine cotyledons are boPAG 56kDa, boPAG 67 kDa and boPAG 75 kDa (Klisch *et al.*, 2005). Bovine PAG-1 polypeptides showed 73 % amino acids identity with ovine PAG1 exhibiting interspecies conservation of PAG-1.

The NH2 terminus of boPAG beginning with Arg-34 suggests further proteolytic processing even after the cleavage of signal peptide. Critical amino acids substitution at the active site of bovine and ovine PAG-1 suggests that the proteins are enzymatically inactive as many possess nucleotide substitutions within the catalytic center (Xie *et al*., 1997). Comparing PAG sequences both within a single species and between species reveals segments of primary structure that are hypervariable and others that are relatively constant. The first 15 amino acids of each PAG molecule which appear to constitute the signal peptide, amino and the carboxyl termini are highly conserved apart from other constant regions. Further a conserved site for EcoR1 is present in every binucleate cell–specific PAG (Garbayo *et al.*, 2008). The conserved regions are ones that are internal and structurally important for retaining the overall three dimensional fold of the molecule (Guruprasad *et al*., 1996). A conserved motif for propeptide removal is the sequence ISF↓RGS, present in all members of the PAG-1 group in contrast to the members of the PAG-2 group, where the site of propepide removal

is difficult to ascertain

Genomic characteristics of Pregnancy Associated Glycoproteins

The PAGs are a multigene family expressed in placenta of eutherian mammals and their expression varies spatially as well as temporally during gestation (Garbayo *et al*., 2008). Recent investigations have demonstrated that different PAG cDNAs are not expressed coordinately throughout pregnancy. Some are expressed early, while others only when pregnancy progresses (Garbayo *et al*., 2000, Green *et al*., 2000, Hughes *et al*., 2000). Screening of placental libraries with nucleic acid probes has identified more than 100 cDNAs that are very abundant and code for polypeptides related but generally distinct from PAGs isolated by biochemical procedure. Multiple PAG genes have been cloned and identified in domestic and wild ruminants as well as in pigs (Xie *et al*., 1991, Szafranska *et al*., 1995; Garbayo *et al*., 2000, Vawter *et al*., 2004).

These identified cDNA sequences represent distinct transcripts encoding unique polypeptide PAG precursors. However, the cDNA of PAGs have yet to be cloned in many species including buffalo. Phylogenetic analyses of PAG and PAG like genes indicate that the PAG family originates from an ancient PAG-like precursor (pro-gene) by duplication and positive selection approximately 86 million years ago (Roberts *et al*.,1996, Hughes *et al*., 2000, 2003), which is consistent with estimates for the split of the artiodactyls from other ungulate orders. The conservation and homology of the PAGs in many eutherians (artiodactyla, perissodactyla, carnivora and rodentia) is suggestive of their importance in the reproductive physiology of ungulate species. Cloning of the PAG and PAG- like gene family revealed distinct chorionic transcripts. The number of the PAG mRNA is known to vary between species and their expression can sometimes change during the course of pregnancy. Presumably, the distinct number and pregnancy-stage dependent expression of the PAG transcripts can be associated with specific requirements of fetal development in different species. The PAG family has several conserved regions and shares nearly 50% sequence identity with the Pepsinogen family. PAG genes have been cloned and sequenced in many species such as cattle, sheep (Xie et al., 1991), goat (Garbayo et al., 2000), pig (Szafranska et al 1995) and white tailed deer (Vawter et al., 2004).

PAG-1 gene

PAG-1 gene was first cloned in cattle followed by sheep, goat and pig. Expression of bPAG-1 was detected by RPA (Ribonuclease Protection Assay) as well as Northern hybridization and it was found that mRNA of the bovine, caprine, ovine and porcine PAG genes are detectable from 15-18 days post- coitum or even before (Xie *et al*., 1991, 1994, 1995, Szafranska *et al*., 1995, Garbayo *et al*., 2000; Green *et al*., 2000, Ushizawa *et al*., 2005).

Table 2. Cloned cDNA of the PAG-1 gene in various species

Name	Accession number	Amino acids	Day of pregnancy	Species	Reference
Buffalo PAG-1	EU815059	380	30	Buffalo	Jerome *et al.* (2011)
Buffalo PAG-2	HM468470	367	-		Green *et al.* (2010)
bPAG-1	M73962 NM17441	380	260	Cattle	Xie *et al.* (1991)
oPAG-1	M73961	382	100	Sheep	
cPAG-1	AF191326	341	45-115	Goat	Garbayo *et al.* (2000)
pPAG-1	L34360	389	13-17	Pig	Szafranska *et al.* (1995)
ePAG	L38511	388	25	Horse	Green *et al.* (1999)
zPAG	AF036952	388	Term placenta	Zebra	
wtdPAG-1	AY509865	380	85-90	White tailed deer	Brandt *et al.* (2007)

Structure of the gene

The identification of the structural organization of the PAG genes or their promoters was performed on the basis of identified gDNA sequences and required single gene isolation by genomic bovine or porcine library screenings (~1.2 kbp promoter and ~ 8 kbp gene).The entire exon-intron structures were identified for two PAG genes only, bovine PAG-1 and porcine PAG-2. The gDNA sequencing of the PAG genes and comparison of the ORF of previously cloned cDNAs allowed structural identification of 9 exons and 8 introns (A-H) in the bovine and porcine PAG genes. In bovine PAG-1 gene, the exons are 99-200 bp long and introns ranges from 85 bp up to ~1.8 kbp. Exon 1 codes for signal peptides and propieces, exons 2-5 code for the N-terminal lobe and exons 6-9 code for the C-terminal lobe of the mature polypeptide (Xie *et al*., 1995). Functional activity of PAG genes begins with the cloning and sequencing of the promoter. So far, only a few promoter sequences of the PAG genes have been identified and deposited in GenBank. The comparison of the PAG promoter sequences revealed that their proximal regions are similar to each other, but not to promoters of other aspartic proteinase or other trophoblast-expressed genes.

A 20 kb genomic fragment, which contained the whole bPAG-1 gene, was isolated from the λGEM -11 genomic libraries (Clone bpg 2000).The transcription start point (tsp) is located 53 or 54 bp upstream from the start codon (ATG) and 19 bp downstream from a 5'-TATATAA sequence. The exon 1 has both noncoding (52 bp) and coding (53 bp) sequence. The latter provides the entire 15 aa signal peptide region and an additional 8 bp encoding for the beginning of the pro-peptide. Exon 2 encodes the remainder of the pro-peptide (aa 1-38) (Xie *et al*., 1995). The mature portion of the protein is coded by the remainder of exon 2, exons 3 to 8 and the first 150 bp of exon 9. A total of 355 bp after the stop codon (TAA) includes a poly-adenylation signal (AATAAA), which is located at the identical position within the bPAG-1 cDNA. The organization of PAG-1 gene is similar to that of all other known aspartic proteinase encoding genes.

Variant of boPAG-1 gene

Placental PAG-1 cDNA was immunoscreened with an anti-bPAG-1 antiserum (Zoli *et al*., 1991). Out of ten immunopositive clones initially purified and partially sequenced, nine matched clone bp314, the cDNA that encodes bPAG-1. However, one clone (bp 111) was distinctly different from the others. The polypeptide encoded by bp111 cDNA has been named bPAG-1var. The two cDNA, bp 111 and bp 314 showed 91.5 and 86.5 % identities at the nucleotide and amino acid sequences, respectively. The mismatches between bPAG-1 and bPAG var are scattered throughout the lengths of the polypeptide, notably aa 76 in bPAGvar is Gly rather than Ala i.e. the difference likely to have inactivated bPAG-1 as a proteinase is not evident in bPAG-1var (Xie *et al*., 1995).

Expression of PAGS during pregnancy

Molecular biology investigations deduced that during certain stages of pregnancy some PAGs were expressed, while others were absent (Garbayo *et al.*

Table 3. Recognized structural organization of boPAG-1 gene deposited in GenBank

Accession number	gDNA sequences	Identified by
L27833	EXONS 1-6	Xie *et al.* (1995)
L22834	EXONS 7-8	
L27832	EXON 9	

Table 4. Identified promoter sequence of PAG-1 gene

Accession number	Length of promoter (bp)	Identified by
L27833	1260	Xie *et al.*, (1995)

Table 5. Cloned cDNA of the PAG gene family of cattle deposited in the GenBank database.

Name	Accession Number	Coded polypeptide (aa)	Transcripts (Day of pregnancy)	Identified by
boPAG-1	M73962	380	260	Xie *et al.* (1991, 1994, 1997)
boPAG-2	NM176614.1	376		
boPAG-3	L06153	381		
boPAG-4	AF020506	380	19-25	
boPAG-5	AF020507	377		
boPAG-6	AF020508	379		
boPAG-7	AF020509	341		
boPAG-8	AF0205010	346		
boPAG-9	AF0205011	381		
boPAG-10	AF0205012	380		
boPAG-11	AF0205013	376		
boPAG-12	AF0205014	375		
boPAG-13	AF192330	370-381	19	Green *et al.* (2000)
boPAG-14	AF192331			
boPAG-15	AF192332			
boPAG-16	AF192333			
boPAG-17	AF192334			
boPAG-18	AF192335			
boPAG-19	AF192336			
boPAG-20	AF192337			
boPAG-21	AF192338			

1999, 2000, Green *et al*., 2000). The PAG molecules which were predominantly expressed in the binucleate cells (like boPAG-1, -6, and -7) were expressed weakly or not in the day 25 placenta, but they were present at the middle and the end of pregnancy. Others like boPAG-4, -5, and -9 were expressed at day 25 and at earlier stages.

By the use of cDNA microarray analysis Ushizawa *et al*. (2005) demonstrated that several PAG molecules are expressed as early as day 7 to 14 of pregnancy boPAG -11,-16 and -17) , days 14 to 21 boPAG -1,-5 to -7,-9 to -13,-15 to -17,-19,-21) or even before (at day 7: boPAG -4,-5 and -6. In species with epitheliochorial placenta, PAGs (eqPAG-1, poPAG-1, and poPAG-2) were reported to be expressed throughout the chorion (Szafranska *et al*., 1995, Green *et al*., 1999).

Functions of PAG

The function of PAG family may be complex and combine with specific expression during pregnancy stages. Some PAG members affect maternal recognition of pregnancy, adhesion of trophoblast and implantation, while other PAG members may be involved in remodeling of the feto-maternal unit during placenta development in various mammalian species. High level of expression in early gestation shows that PAG may be involved in implantation, maternal recognition of pregnancy, placentogenesis and placental modeling. PAG has also been implicated to have luteotrophic, luteoprotective and regarded as a good indicator of embryo viability (Szafranska *et al.*, 2007). PAG proteins were hypothesized as local immunosuppressive effect that can be involved in the maintenance of the histoincompatible feto-maternal unit (Dosogne *et al*., 2000).The concentration of PAG in maternal circulation depends on the number of the well being embryos/fetuses, higher in twin bearing females (Mialon *et al*., 1993, Willard *et al*., 1995, Batalha *et al*., 2001). The secretion of PAG also differs based on the sex and the breed of the fetus (Zoli *et al*., 1992).

Potential role of PAG as pregnancy diagnostic marker

Purified native or recombinant PAG proteins and specific anti-PAG sera has led to the establishment of several diagnostic tests for early pregnancy diagnosis based on the detection of PAGs in maternal blood plasma or serum and provide an alternative method for determining the embryonic survival (Szenci *et al*., 1998, 2003). The major significance of PAG assays in ruminants, similar to urine or plasma hCG tests in primates, done by RIA and ELISA system is the direct identification of a placental product present in the maternal system that can be used as a marker of a viable pregnancy and embryonic mortality in various ruminants (Zoli *et al*., 1992, Humblot, 2001, Szenci *et al*., 2003, Gonzalez *et al*., 2004). These glycoproteins could be detected in the maternal circulation at around the time when the trophoblast forms definitive attachment to the uterine wall (Sasser *et al*., 1986; Zoli *et al*., 1992). The measurement of PAG protein concentrations in maternal blood or milk of ruminants allow for 76.6 -100% accuracy rates for early pregnancy diagnosis (Gonzalez *et al*., 2001, 2004). The mean concentration of PAG in pregnant cattle is between 0.5-0.8 ng/ml. The half-life of PAG was estimated to be 7.4 to 9 days (Kiracofe *et al*., 1993; Mialon *et al*., 1993; Ali *et al.*, 1997). PAG has been reported to be pregnancy stage dependent; it increases as pregnancy progresses (Green *et al*., 2005). The homologous and heterologous PAG assays allow for early pregnancy diagnoses in domestic cattle (Sasser et al., 1986, El Amiri *et al*., 2000, Humblot, 2001, Green *et al*., 2005), sheep (Ranilla et al., 1994, Gajewski *et al*., 1999), goats (Gonzalez *et al*., 1999), white tailed deer (Osborn *et al*., 1996) and other wild ruminants (Huang *et al*., 1999). Heterologous RIA systems such as RIA 706 and RIA 708 have been developed by Perenyi *et al*. (2002) with antisera raised against PAG molecules isolated from caprine placenta. These RIA systems have been found to be more sensitive and specific than homologous RIA. Sandwich ELISA has been developed between day 24 and 28 of pregnancy by using semi purified PAG proteins (Green et al., 2000).Bovine PAG RIA as compared to other methods of early pregnancy diagnosis has been reported to be more sensitive as well as specific (Skinner *et al*., 1996). A commercial ELISA kit using purified PAG-1 has been developed with the name BioPRYN by Biotracking Company (USA). It has been found successful in detecting pregnancy by day 28 post-insemination having a success rate of 97%.

Research on PAG in buffaloes

Attempts have been made to isolate and purify pregnancy associated glycoproteins in buffalo but only partial purification could be achieved (Barbato *et al*., 2003, Singh *et al*., 2005). Singh *et al.* (2005) isolated six proteins from buffalo placental extract with molecular weights of 78, 67, 53, 42, 33 and 26 kDa. Dot ELISA developed to diagnose pregnancy by using hyper immune sera against these partially purified proteins showed a success rate of 68%. Protein sequence with the name of PAG75_BUBBU (P85048) has been submitted by Barbato *et al*. (2006). Recently, Barbato *et al*. (2008) isolated and purified distinct buffalo PAG proteins by Vicia villosa agarose affinity chromatography. Western blotting with anti-PAG sera showed that the apparent molecular masses of the immunoreactive bands from the Vicia villosa agarose peaks range from 59.5 to 75.8 kDa and from 57.8 to 73.3 kDa from the midpregnancy and late pregnancy placentas, respectively. Amino-terminal microsequencing of these immunoreactive proteins has allowed the identification of three distinct buffalo PAG sequences, which have been deposited in the SwissProt database as RGSXLTIHPLRNIRDFFYVG (Acc. no. P85048), RGSXLTILPLRNIID (Acc. no. P85049) and RGSXLTHLPLRNI (Acc. no. P85050). Karen et al. (2007) studied PAG concentration using heterologous double antibody RIA for diagnosis of pregnancy in buffalo between days 19 and 55 post-breeding. The sensitivity of PAG-RIA test was 11.1% at days 19-24 and reached 100% from day 31 after breeding. The specificity of test ranged from 90 to 100% from 19 to 55 days post-breeding, indicating PAG-RIA test as highly accurate for detecting pregnancy in buffaloes from day 31 onwards after breeding. Genomic study on buffalo PAG carried by Jerome *et al*. 2011 revealed buffalo PAG-1 gene possess conserved regions and key mutations similar to bovine rendering them proteolytically inactive. It is evident that expression of buffalo PAG-1 gene was throughout the pregnancy starting from day 30 to term (Jerome *et al.* 2011b). Molecular modeling studies of buffalo PAG-1 re-

vealed its bilobed structure with pepstatin binding clefts near the active region (Jerome et al. 2011c).

Conclusion

In conclusion, optimum reproductive management in domestic animals is possible by accurate pregnancy diagnosis which in turn depicts animals, fertility. With the advent of 'omics' research viz. genomic, proteomic, transcriptomics such endeavors will result in the development of novel pregnancy biomarkers and their diagnostics.

References

Ali, M., Gebbie, F.E., Sinclair, K.D., Hutchinson, J.S.M., Beckers, J.F., Broadbent, P.J., 1997. Half-life of serum bovine Pregnancy-Associated Glycoprotein (bPAG), post-partum anoestrus and performance of beef cows. Journal of Reproduction and. Fertility Abstract series 20, p 25.

Atkinson, Y.H., Gogolin –Ewens, K.J., Hounsel, E.F., Davies, M.J., Brandon, M.R., Seamark, R.F.,1993. Characterization of placentation-specific binucleate cell glycoproteins possessing a novel carbohydrate. Journal of Biological Chemistry 268, 26679-26685.

Ayad, A., Sousa, N.M., Sulon, J., Iguer-Ouada, M., Beckers, J.F., 2007. Comparision of five radioimmunoassay systems for PAG measurement: ability to detect early pregnancy in cows. Reproduction in Domestic Animals 42(4), 433-440.

Barbato O,. Sousa N.M,. Klisch K,. Clerget E,. Debenedetti A,. Barile V.L,. Malfatti A., Beckers. J.F., 2008 Isolation of new pregnancy-associated glycoproteins from water buffalo (Bubalus bubalis) placenta by Vicia villosa affinity chromatography. Research in Veterinary Science 85(3), 457-66.

Barbato, O., Sousa, N. M., Klisch, K.,2006. Isolation of Pregnancy-Associated Glycoproteins from water buffalo (Bubalus bubalis) placenta by Vicia villosa affinity Chromatography. UniProt KB/-Prot P85048.

Barbato, O., Sousa, N. M., Malfatti, A., Beckers, J. F., De Santis, G., Debenetti, A., 2003. Purification of Pregnancy-associated glycoproteins (PAGs) in buffalo (Bubalus bubalis): Preliminary results. In: Atti II Congresso Nazionale sull'Allevamento del Bufalo,Roma, Italy. pp 279-283.

Bartol, F. F., Roberts, R. M., Bazer, F. W., Lewis, G.S., Godkin, J. D., Thatcher, W.W., 1985. Characterization of proteins produced in vitro by peri-attachment bovine conceptus. Biology of Reproduction 32, 681-693.

Batalha, E. S., Sulon, J., Figueiredo, J. R., Beckers, J.F., Espeschit, C.J.B., Martins, R., Silva, L.D.M., 2001. Plasma profile of pregnancy associated glycoprotein (PAG) in pregnant Alpine goats using two radioimmunoassay (RIA) systems. Small. Ruminant Research 42, 111-118.

Beckers, J.F., De Coster, R., Wouters-Ballman, P., Fromont Ienard,C., Vanderz Walmen, P., Ectors, F., 1982. Dosage radioimmunologique de l'hormone placentaire somatotrope et mammotrope bovine. Annals of Veterinary Medicine 126, 9.

Brandt, G.A., Parks, T.E., Killian, G., Ealy, A.D., Green, J.A., 2007. A cloning and expression analysis of pregnancy-associated glycoproteins expressed in trophoblasts of the white-tail deer placenta. Molecular Reproduction Development 74(11), 1355-1362.

Butler, J.E., Hamilton, W.C., Sasser, R.G., Ruder, C.A., Hass, G .M., Wiliams, R.J., 1982. Detection and partial characterization of two bovine Pregnancy-Associated Proteins. Biology of Reproduction 26, 925−933.

Byatt, J.C., Wallace, C.R., Bremel, R.D., Collier, R.J., Bolt, D. J., 1987. The concentration of bovine placental lactogen and the incidence of different forms in fetal cotyledons and in fetal serum domes. Domestic Animal Endocrinology 4, 231-41.

Byatt, J.C., Shimomura, K., Duello, T.M., Bremel, R.D., 1986. Isolation and Characterization of multiple forms of bovine placental lactogen from secretory granules of the fetal cotyledon. Endrocrinology 119,1343-50.

Byatt, J.C., Warren, W.C., Eppard, P.J., Staten, N.R., Krivi, G.G., Collier, R.J., 1992. Ruminant placental lactogens - structure and biology. Journal of Animal Science 70, 2911-2923.

Cole, H.H., Hart, G.H., 1930. The potency of blood serum of mares in progressive stages of pregnancy in effecting the sexual maturity of the immature rat. American Journal of Physiology 93, 57-68.

Dosogne, H., Massart-Leen, A. M., Bervenich, C., 2000. Immunological aspects of pregnancy associated glycoproteins. Advanced Cell Biology 480, 295−305.

El Amiri, B., Remy, B., De Sousa, N. M., Joris, B., Ottiers, N. G., Perenyi, Z., Banga-Mboko, H., Beckers, J. F., 2003. Isolation and partial characterization of three pregnancy-associated glycoproteins from the ewe placenta. Molecular Reproduction Development 64, 199-206.

El Amiri, B., Sousa, N. M., Perenyi, Z. S., Banga-Mboko, H., Beckers, J. F., 2000. Pregnancy-associated glycoproteins (PAG) in Bos taurus taurus and Bos taurus indicus. Theriogenology 53, 281-283.

El miri, B .,Sulon, J., Karen, A., 2007. Development and validation of two homologous radioimmunoassays for measuring pregnancy associated glycoprotein (PAG) concentration in ewe. Reproduction in Domestic Animal 42(3), 257-62.

Gajewski, Z., Beckers, J .F., Melo De Sousa, N.,Thun, R., Sulon, J.,Faundez, R., 1999. Determination of pregnancy associated glycoprotein concentrations in sheep, a retrospective study. Advanced Cell Biology 26, 89-96.

Garbayo, J.M., Green, J.A., Manikkam, M., Beckers, J.F., Kiesling, D.O., Ealy, A.D., Roberts, M., 2000. Caprine pregnancy-associated glycoproteins (PAG): their cloning, expression, and evolutionary relationship to other PAG. Molecular Reproduction Development 57, 311-322.

Garbayo, J.M., Remy, B., Alabart, J.L., Folch, J., Wattiez, R., Falmagne, P., Beckers, J.F., 1998. Isolation and par-

tial characterization of a pregnancy-associated glycoprotein (PAG) family from the goat placenta. Biology of Reproduction 58, 109-115.

Garbayo, J.M., Serrano.B., Lopez-Gatius.F., 2008. Identification of novel PAGs expressed by the peri-implantation conceptus of domestic ruminants. Animal . Reproduction. Science 103, 120-134.

Gonzalez, F., Cabrera, F., Batista, M., Rodriguez, N., Alamo, D., Sulon, J., Beckers J. F., Gracia, A., 2004. A comparison of diagnosis of pregnancy in the goat via transrectal ultrasound scanning, progesterone, and pregnancy-associated glycoprotein assays. Theriogenology 62, 1108–1115.

Gonzalez, F., Sulon, J., Garbayo, J. M., 1999. Early pregnancy diagnosis in goats by determination of pregnancy-associated glycoprotein concentrations in plasma samples. Theriogenology 52, 717-725.

Gonzalez, F., Sulon, J., Calero, P., Batista, M., Gracia, A., Beckers, J. F., 2001. Pregnancy associated glycoproteins (PAG) detection in milk samples for pregnancy diagnosis in dairy goats. Theriogenology 56, 671−676.

Green, J. A., Parks, T. E., Avalle, M. P., 2005. The establishment of an ELISA for the detection of pregnancy-associated glycoproteins (PAGs) in the serum of pregnant cows and heifers. Theriogenology 63, 1481-1503.

Green, J.A, Xie, S., Quan, X., Bao, B., Gan, X., Mathialagan, N., Beckers, J.F., Roberts, R.M., 2000. Pregnancy-associated bovine and ovine glycoproteins exhibit spatially and temporally distinct expression patterns during pregnancy. Biology of Reproduction 62, 1624-1631.

Green, J.A, Xie, S., Szafranska, B., Gan, X., Newman, A.G., McDowell, K., Roberts, R.M., 1999. Identification of a new Aspartic Proteinase expressed by the outer chorionic cell layer of the equine placenta. Biology of Reproduction 60, 1069-1077.

Guruprasad, K., Blundell,T.L., Xie,S., Green,J., Szafranska,B., Nagel,R.J., McDowel,K., Baker,C.B., Roberts, R.M.,1996 Protein Engineering 9, 849-856.

Huang, F., Cockrell, D.C., Stephenson, T.R., Noyes, J.H., Sasser, R. G., 1999. Isolation, purification, and characterization of pregnancy-specific protein B from elk and moose placenta. Biology of Reproduction 61, 1056-1061.

Humblot, P., 2001. Use of pregnancy specific proteins and progesterone assays to monitor pregnancy and determine the timing, frequencies and sources of embryonic mortality in ruminants. Theriogenology 56, 1417−1433.

Humblot, P., Camous, S., Martal, J., 1988. Diagnosis of pregnancy by radioimmunoassay of a pregnancy-specific protein in the plasma of dairy cows. Theriogenology 30, 257-268.

Jerome A., S. K. Singh, S. K. Agarwal, Mohini Saini.,Ashwin Raut., 2011 Characterization and In Silico Analysis of Pregnancy-Associated Glycoprotein-1 Gene of Buffalo (Bubalus bubalis). Genetics Research International doi:10.4061/2011/436138

Jerome. A, Singh. S.K, Agarwal. S.K, Sarath. T., Arunmozhi. N., 2011b Detection of buffalo Pregnancy Associated Glycoprotein-1 gene transcripts at different stages of pregnancy. Indian Journal of Animal Reproduction, (Accepted Manuscript in press)

Jerome, A., Singh, S.K., Agarwal, S.K., 2011c. Agarwal Structural modeling and analysis of Pregnancy Associated Glycoprotein-1 of buffalo (Bubalus bubalis).ISRN Molecular Biology (Accepted Manuscript in press) .

Kappes, S.M., Warren, W.C., Pratt, S.L., Liang, R., Anthony, R.V., 1992. Quantification and cellular localization of ovine placental messenger ribonucleic acid expression during mid and late gestation. Endocrinology 131, 2829-2838.

Karen, A.,Darwish, S., Ramoun, A., Tawfeek, K., Van Hanh, N.,De Sousa, M ., Sulon, J., Szenci, O., Beckers, J .F., 2007. Accuracy of ultrasonography and pregnancy – associated glycoprotein test for pregnancy diagnosis in buffaloes. Theriogenology 68(8), 1150-55.

Kiewisz, J., Sousa,N.M., Beckers, J.F., Vervaecke, H., Panasiewicz, G., Szafranska, B., 2008. Isolation of pregnancy-associated glycoproteins from placenta of the American bison (Bison bison) at first half of pregnancy. General and comaperative Endocrinology 155(1), 164-75.

Kiewisz, J., Sousa, N. M., Beckers, J.F., Panasiewicz, G., Gajewski, Z., Szafranska, M., 2009. Identification of multiple pregnancy-associated glycoproteins (PAGs) purified from the European bison (Bison bonasus) placentas. Animal Reproduction Science 112(3-4), 229-50.

Kiracofe, G.H., Wright, J.M, Schalles, R.R., Ruder, C.A., Parish, S., Sasser, R.G., 1993. Pregnancy-specific protein B in serum of postpartum beef cows. Journal of Animal Science 71(8), 2199-2205.

Klisch, K., De Sousa, N. M., Beckers, J. F., Leiser, R., Pich, A., 2005. Pregnancy associated glycoprotein-1,-6,-7 and -17 are major products of bovine binucleate trophoblast giant cells at mid pregnancy. Molecular Reproduction Development 71, 453−460.

Knickerbockers, J.J., Thatcher, W.W., Bazer, F.W., Drost, M.,Barron, D.H., Finher, K.B., Roberts, R.M., 1986. Proteins secreted by day-16 to 18 bovine conceptuses extend corpus luteum function in cows. Journal of Reproduction and Fertility 77, 381-391.

Mialon, M. M., Camous, S., Renand, G., Martal, J., Menissier, F., 1993. Peripheral concentrations of a 60-kDa pregnancy serum protein during gestation and after calving and in relationship to embryonic mortality in cattle. Reproduction Nutrition Development 33, 269−282.

Mialon, M.M., Renand, G., Camous, S., Martal, J., Menissier, F., 1994. Detection of pregnancy by radioimmunoassay of a pregnancy serum protein (PSP60) in cattle. Reproduction Fertility Development 34, 65-72.

Osborn, D. A., Beckers, J. F., Sulon, J., Gassett, J.W., Muller, L.I., Murphy, B P., Miller, K.V., Marchinton, R. L., 1996. Use of glycoprotein assays for pregnancy diagnosis in white-tailed deer. Journal of Wildlife Management 60, 388−393.

Perenyi, Z., Szenci, O., Sulon, J., Drion, P. V., Beckers, J .F.,

2002. Comparison of the ability of three radioimmunoassays to detect pregnancy-associated glycoproteins in bovine plasma. Reproduction in Domestic Animals 37, 100-104.

Ranilla, M.J., Sulton, J., Carro, M.D., Mantecon, A.R., Beckers, J.F., 1994. Plasma profiles of pregnancy-associated glycoprotein and progesterone levels during gestation in Churra and Merino sheep. Theriogenology 42, 537−545.

Roberts, R.M., Chen, Y., Ezashi, T., Walker, A.M., 2008. Interferons and the maternal-conceptus dialog in mammals. Seminars in Cell and Developmental Biology 19(2), 170-177.

Roberts, R.M., 1996. Interferon-tau and Pregnancy. Journal of Interferon and Cytokine Research 16(4), 271-273.

Sasser, R. G., Ruder, C. A., Ivani, K. A., Butler, J. E., Hamilton, W.C., 1986. Detection of pregnancy by radioimmunoassay of a novel pregnancy-specific protein in serum of cows and a profile of serum concentrations during gestation. Biology of Reproduction 35, 936-942.

Silva, E., R.A. Sterry, D. Kolb, N. Mathialagan, M.F. McGrath, J.M. Ballam, Fricke P. M., 2007. Accuracy of a Pregnancy-Associated Glycoprotein ELISA to Determine. Pregnancy Status of Lactating Dairy Cows Twenty-Seven Days after Timed Artificial Insemination. Journal of Dairy Science 90, 4612–4622.

Singh, S.K., Agarwal, S.K., Shankar, U., Gupta, L., 2005. Purification and Characterization of protein(s) from placental extracts in buffalo. Indian Journal of Animal Science 75 (7) ,769-72.

Skinner, J.G., Gray, D., Gebbie, F.E., Beckers, J.F., Sulon, J., 1996. Field evaluation of pregnancy diagnosis using bovine pregnancy associated glycoprotein (bPAG). Cattle Practice4, 281-284.

Sousa, N. M., Ayad, A., Beckers, Z., Gajewski, Z.,2006. Pregnancy Associated Glycoprotein as pregnancy markers in the ruminants. Journal of Physiology and Pharmacology 57, (Supp 8), 153-171.

Sousa, N.M., Remy, B., El Amiri, B., De Figueiredo, J.R., Banga-Mboko, H., Dias Goncalves, P. B., Beckers, J.F., 2002. Characterization of pregnancy-associated glycoproteins extracted from Zebu (Bos indicus) placentas removed at different gestational periods. Reproduction Nutrition Development 42, 227−241.

Spencer, T.E., Burghardt, R.C., Johnson, G.A., Bazer, F.W., 2004. Conceptus signals for establishment and maintenance of pregnancy. Animal Reproduction Science 82,537-550.

Szafranska, B., Panasiewicz, G., Majewska, M., 2006. Biodiversity of multiple pregnancy –asociated glycoprotein (PAG) family: Gene cloning and chorionic protein purification in domestic and wild Eutherians (Placentalia) – a review. Reproduction Nutrition Development 5,481-502.

Szafranska, B., Panasiewiez, G., Majewska, M., Romanowska, A., Dajnowiee, J., 2007. Pregnancy Associated Glycoprotein family - as chorionic signaling ligands for gonadotropin receptors of cyclic animals. Animal Reproduction. Science 99, 269-284.

Szafranska, B., Xie, S., Green, J., Roberts, R.M., 1995. Porcine Pregnancy-Associated Glycoproteins: new members of the aspartic proteinase gene family expressed in the trophectoderm. Biology of Reproduction 53, 21−28.

Szenci, O., Beckers, J. F., Humblot, P., Sasser, G.,Taverne, M.A., Varga, J., Baltusen, R., Schekk, G., 1998. Comparison of ultrasonography, bovine pregnancy-specific protein B, and bovine pregnancy-associated glycoprotein 1 tests for pregnancy detection in dairy cows. Theriogenology 50, 77−88.

Szenci, O., Beckers, J. F., Sulon, J., Bevers, M.M., Borzsonnyi, L., Fodor, L., Kovacs, F., Taverne, M. A. M., 2003. Effect of induction of late embryonic mortality on plasma profiles of pregnancy associated glycoproteins 1 in heifers. Veterinary Journal 165, 307-313.

Ushizawa, K., Herath, C.B., Kaneyama, K., 2004. cDNA microarray analysis of bovine embryo gene expression profiles during the pre-implantation period. Reproductive Biology and Endocrinology 2, 77.

Willard, S.T., White, D.R., Wesson, C.A.R.,Stellflug, J., Sasser, R.G., 1995. Detection of fetal twins in sheep using radioimmunoassay for pregnancy-specific protein B. Journal of Animal, Science 73, 960−966.

Xie, S., Green, J., Bao, B., Beckers, J. F., Valdez, K. E., Hakami, L., Roberts, R.M.,1997. Multiple pregnancy-associated glycoproteins are secreted by day 100 ovine placental tissue. Biology of Reproduction 57, 1384−1393.

Xie, S., Low, B. G., Nagel, R. J., Kramer, K. K., Anthony, R. V., Zoli, A. P., Beckers, J. F., Roberts, R. M., 1991. Identification of the major pregnancy specific antigens of cattle and sheep as inactive members of the Aspartic Proteinase family. Proceedings of the National Academy of Sciences of the United States of America 88,10247−10251.

Zoli, A. P., Guilbault, L. A., Delabaut, P., Orates, W. E .B., Beckers, J. F., 1992. Radioimmunoassay of a bovine pregnancy-associated glycoprotein in serum: its application for pregnancy diagnosis. Biology of Reproduction 46, 83-92.

Zoli, A.P., Beckers, J.F., Wouters-Ballman, P., Closset, J., Falmagne, P., Ectors, F., 1991. Purification and characterization of a bovine pregnancy-associated glyoprotein. Biology of Reproduction 45, 1-10.