

# EXPRESSION OF PREGNANCY ASSOCIATED GLYCOPROTEINS (PAGs) DURING DIFFERENT STAGES OF PREGNANCY - A MARKER FOR EARLY PREGNANCY DIAGNOSIS IN BUFFALOES

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ABSTRACT

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The pregnancy associated glycoproteins (PAGs) are a complex group of proteins secreted from the mono- and bi-nucleate cells of the trophoblastic epithelium that have been used as early markers of pregnancy in bovines with varying accuracies. This experiment was designed to study the expression of PAG genes at different stages of pregnancy in buffalo. The PAG-1 and PAG-2 genes were identified as early markers of pregnancy at the feto-maternal interface in the pregnant uteri of buffaloes obtained from local abattoir and were found to be expressed from one to four month of pregnancy. In conclusion, PAGs could be used as one of the accurate markers of early pregnancy diagnosis in buffaloes.

**Keywords:** Glycoproteins, Pregnancy diagnosis, Buffaloes

Diagnosis of early pregnancy is crucially needed to recognize non pregnancy at the earliest to identify open animals so as to treat or to rebreed them at the earliest possible time. Lack of reliable early pregnancy diagnosis methods in buffaloes aggravates the problems of increased calving interval and infertility. Non-invasive methods currently available are specific such as ultrasonography, but needs sophisticated instruments and technically skilled persons. However, Whitlock and Maxwell (2008) reviewed that most of the embryonic losses occurred during the first few days after fertilization and during the process of implantation and became less frequent beyond 50 days.

Hence, the study on the expression pattern of different early biological indicators of pregnancy particularly PAGs could bring about the solution for the diagnosis of early embryonic and fetal mortalities (Barbato and Barile, 2012). Studies on levels of progesterone, pregnancy associated glycoproteins (PAGs), interferon tau, and early pregnancy factor are

some of the common clinically practiced pregnancy detection methods in bovines, and each has its own benefits and limitations (Balhara *et al.*, 2013). Hence the present experiment was designed to study the PAG 1 expression pattern during different stages of pregnancy in buffaloes.

## MATERIALS AND METHODS

The expression pattern of PAG I was studied in placentomes at different stages of pregnancy from 18 gravid buffalo uteri of different gestational age collected from Chennai slaughter house and processed in a sterile manner. The crown rump (CR) length of the fetus was measured from the frontal bone to the base of the tail or it was the least straight distance measured from the inner canthus to the base of the tail. CR length in inches multiplied by 2 and the resultant square root gives the approximate gestational age in months (Singh *et al.*, 1963).

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Based on CR length of conceptus, the stages of pregnancy were identified and the experimental groups were categorized as Group I: 20 to 30 days pregnant, Group II: 30 to 60 days pregnant and Group III: 60 days and above pregnant. The placentomes were collected from gravid uterii (18 nos. 6 nos. in each group) in sterile container.

Total RNA was isolated from the maternal caruncular tissues and fetal cotyledons separately using TRI reagent (Ambion, USA) followed by DNase treatment. The integrity of the extracted RNA was checked by visualizing typical RNA band patterns by running agarose gel electrophoresis. cDNA was synthesized as per standard protocol described in readymade RT (MBI Fermentas) kit. Optimization of PCR reaction of buffalo PAG 1 was achieved using the designed primers F – 5'GTT CGG ATT TGG CAA CTT GT3' and R – 5'GTA GTA GGC CCC ACC AAA CA3'. The amplified product was visualized by running 1% agarose gel in 1 x TAE buffer along with 100 bp DNA marker and documented using Gel Documentation system – XLR (Bio-Rad, USA). The expression pattern of PAG 1 was studied finally.

## RESULTS AND DISCUSSION

Based on CR length the age of the fetuses was measured as less than one month, one to two month and above three month of pregnancy period. Agarose gel electrophoresis of the amplicons of PAG 1 gene revealed that expression of PAG 1 gene (246 bp) was detectable in maternal caruncular tissues and foetal cotyledons. Agarose gel electrophoresis of the resultant amplicons revealed bright bands corresponding to 246 bp in both maternal caruncular and fetal cotyledons. Expression of PAG 1 gene was

recorded in all the three gestational groups studied. (Fig. 1 and 2).

The pregnancy associated glycoproteins (PAGs) are secretory products from the mono- and bi-nucleated trophoblastic cells in bovine placentomes. PAGs are complex group of proteins, a fact proven by the already documented 22 distinct cDNA libraries (Constanta *et al.*, 2011). Pregnancy associated glycoproteins are separated into two groups (Wooding *et al.*, 2005) first being largely localized to the placental feto-maternal interface has been designated as PAG 2 and a separate subgroup expressed primarily in trophoblastic binucleate cells has been designated as PAG 1 (Whitlock and Maxwell, 2008). Among all these PAGs, the three most studied bovine PAGs PSPB, PAG 67 kDa or bPAG-1 (Xie *et al.*, 1994) and PSP60 (Mialon *et al.*, 1993) are isomers of the same protein having similar N-terminal sequences. Transcription of certain PAGs are pregnancy stage or time specific i.e. bPAG 2 and 11 mRNA is seen all through the pregnancy 4, 5, and 9 mRNAs in early pregnancy and bPAG 1 mRNA is detectable only after day 45. Interestingly, bovine PAG 4 and bPAG 1 mRNA are highly transcribed till day 250 of gestation but become indiscernible at the end (Green *et al.*, 2000). In the present study, PAG 1 gene was found to be expressed both in maternal and foetal cotyledons of pregnancy about one month to four month of gestation. According to Xie *et al.* (1997) these PAGs have six N-glycosylation sites, hence responsible for the variations in molecular weight and half-life of PAGs (Klisch *et al.*, 2006) and this might also be the reason for expression of different PAGs during different stages of gestation (Telugu *et al.*, 2009). Hence the present study summarizes that PAGs could be used as a technique of pregnancy diagnosis as early as from one month to four months of gestation in buffaloes.



**Fig. 1. Agarose gel electrophoresis of PAG 1 gene expression in maternal caruncular tissues**

Lane 1 – PAG 1 in Group – I (20-30 days pregnancy)

Lane 2 – PAG 1 in Group – II (30-60 days pregnancy)

Lane 3 – PAG 1 in Group – III (60 days and above of pregnancy)

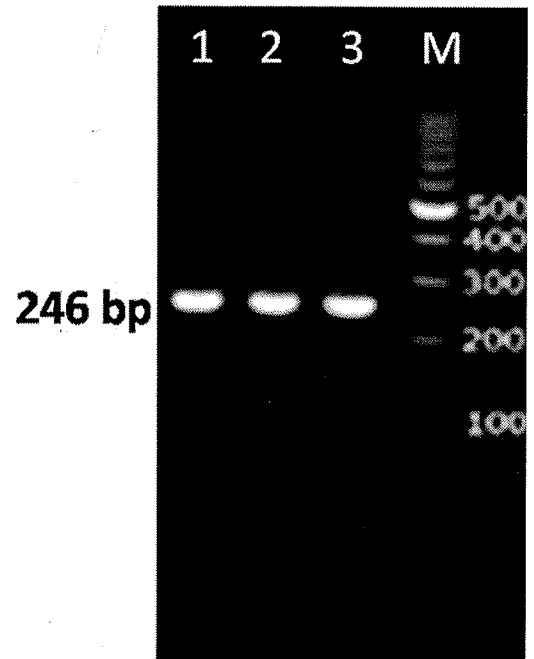
Lane M - 100 bp DNA ladder

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**Fig. 2. Agarose gel electrophoresis of PAG 1 gene expression in foetal cotyledons**

Lane 1 – PAG 1 in Group – I (20-30 days pregnancy)

Lane 2 – PAG 1 in Group – II (30-60 days pregnancy)

Lane 3 – PAG 1 in Group – III (60 days and above of pregnancy)

Lane M - 100 bp DNA ladder

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