DETECTION OF BUFFALO PREGNANCY ASSOCIATED GLYCOPROTEIN-1 GENE TRANSCRIPTS AT DIFFERENT STAGES OF PREGNANCY

JEROME. A *[1], SINGH. S. K, AGARWAL. S. K, SARATH. T [2] AND ARUNMOZHI. N [3]

Division of Animal Reproduction, Indian Veterinary Research Institute, Izatnagar, UP - 243 122

Received : 13.12.2010 Accepted : 14.09.2011

ABSTRACT

Pregnancy is established and maintained in response to a series of interactions between the conceptus and maternal reproductive tract through a large family trophoctoderm proteins i.e. Pregnancy associated glycoprotein. PAG-1 gene has been characterized and its expression has been detected in many domestic, wild ruminants as well as pigs but not in buffalo. In this study buffalo PAG-1 gene transcripts was PCR amplified during various stages of pregnancy i.e. (Day 30, 50, 60, 70, 90, 110, 150 and full term). Moreover buffalo PAG-1 gene was characterized using *EcoR* É as restriction enzyme showed the presence of the conserved site for *EcoR* I for every binucleate specific PAGs especially PAG-1. From the present study, it was found that buffalo Pregnancy Associated Glycoprotein-1 gene was expressed throughout gestation which could be detected qualitatively confirming the expression of the buffalo Pregnancy Associated Glycoprotein-1 gene transcripts throughout pregnancy similar to cattle and other species.

Key words: PAG 1, Buffalo, Gene expression, Pregnancy

INTRODUCTION

Pregnancy is established and maintained in response to a series of interactions between the conceptus and maternal reproductive tract. These interactions between the conceptus and maternal system is established by various cytokines, hormones etc., both from dam and conceptus. (Roberts *et al.*, 2008). The Pregnancy associated glycoproteins (PAGs) are one such large family of proteins which are expressed abundantly and secreted from the placental trophoctoderm during pregnancy. Pregnancy Associated Glycoprotein-1 (PAG-1) is secreted by the binucleate cells of the conceptus at various stages of gestation (Green *et al.*, 1999) and their secretion is regulated by the spatial expression of PAG-1 gene (Patel *et al.*, 2004).

1,*Corresponding author: Scientist, Buffalo Physiology and Reproduction Division, Central Institute for Research on Buffaloes, Hisar, Haryana-125001; [2&3] Assistant professors, Dept. of ARGO, Madras Veterinary College, Chennai-7

MATERIALS AND METHODS

The present study was conducted to detect and characterize binucleate cell specific Pregnancy Associated Glycoprotein-1 (PAG-1) gene transcript during various stages of pregnancy in buffalo. Pregnant female genitalia were obtained from the local slaughter house and the fetuses along with the fetal membranes were removed. The age of the fetus was determined by measuring the crown–rump length (Richardson, 1980). Total cellular RNA from Fetal cotyledons from different stages of pregnancy (Day 30, 50, 60, 70, 90, 110, 150 and full term) was isolated. Total RNA was isolated from the fetal cotyledons using TRI reagent (Ambion, USA). Approximately 100 mg of tissues was homogenized in 1.0 ml of TRI reagent using pestle and mortar. The resultant homogenate was centrifuged at 12000 X g for 15 min at 4°C. The upper aqueous phase rich in RNA was precipitated using 0.5 ml of isopropanol per ml of TRI reagent. The RNA pellet was obtained after centrifugation at 12000 X g for 10 min at 4°C. Finally the pellet was dissolved in 20 µl milliq water and stored

at -20°C till use. The spectrometric absorbance value and the concentration of the obtained RNA (A_{260}/A_{280}) was found to be 1.8 and 1.20 µg per gram of tissue, respectively indicated the high purity of the RNA and absence of contamination with protein and DNA. The integrity of extracted RNA was checked by visualizing typical RNA band patterns by running agarose gel electrophoresis and visualized under gel documentation system. cDNA was synthesized by incubating the obtained RNA, dNTPs, oligo-dT primer, RNase inhibitor and reverse transcriptase at 37°C for 1 hr.

The product (cDNA) was confirmed by amplifying â-actin gene as a positive control. Amplification of â-actin gene of 950 bp was also done every time and considered as a positive control. Buffalo PAG-1 gene transcripts were detected by using PCR primers forward primer PAG-1: 5'-GGATCCAGGAAATAAACATGAAGTG-3', reverse primer PAG-1: 5'-TTACTGAACCACTCYMAGCATTT-3' with PCR reaction mixture with Mg (2.0 mM), dNTPs (200 µM), primers pairs (5 pM) and *Taq* DNA polymerase (1.0 U). Each amplification cycle consisted of denaturation at 94°C for 15 sec, annealing temperature at 51.3° C for 45 sec, extension at 72°C for 45 sec and one cycle of final extension at 74°C for 10 min.

RESULTS AND DISCUSSION

Following standard PCR protocol buffalo PAG-1 gene transcripts were detected from placental samples obtained from day 30, 50, 60, 70, 90, 110, 150 and full term of pregnancy as a single specific band of 1181 base pairs (Fig.1). This is in accordance with the reports of binucleate cells specific PAGs particularly PAG-1 being expressed during different stages of pregnancy starting as early as day 30 in cattle (Garbayo *et al.* 2000; Green *et al.* 2000). Characterization of PAG-1 gene was done using enzyme *EcoR* I É as restriction enzyme. Restriction analysis of the PCR products of each stage with *EcoR* É divided into fragments of 234 and 947 bp, confirming the presence of a conserved site for *EcoR1* for every binucleate specific PAGs especially PAG-1 (Garbayo *et al.*, 2008). From this study detection and characterization of buffalo PAG-1 gene transcripts during different stages of pregnancy was done for the first time. Moreover it was found that buffalo Pregnancy Associated Glycoprotein-1 gene was expressed throughout gestation similar to cattle which could be detected qualitatively. (Xie *et al.*, 1995).

REFERENCES

Garbayo, J.M., Serrano.B. and Lopez-Gatius.F (2008). Identification of novel PAGs expressed by the peri-implantation conceptus of domestic ruminants. *Anim. Reprod. Sci.*, **103**: 120-134.

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

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

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

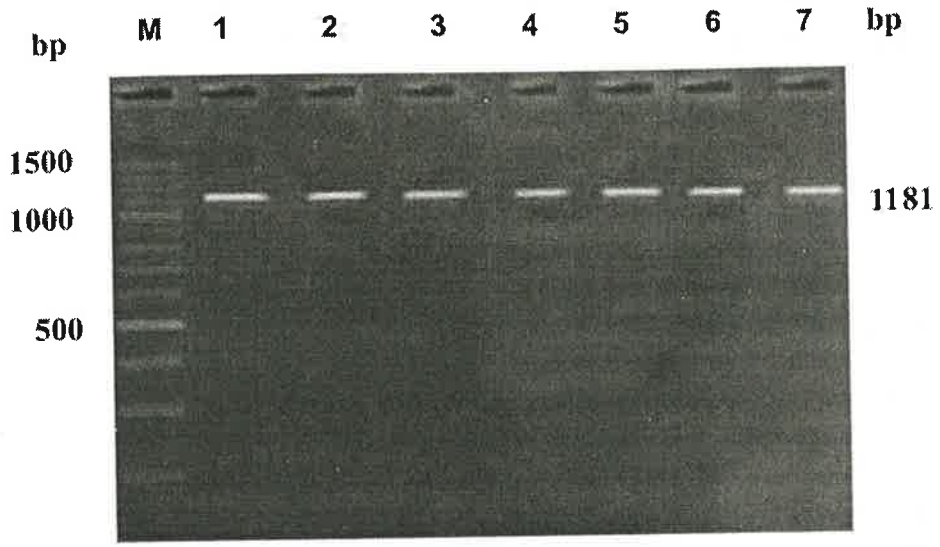
Patel, O. V., Takahashi, T., Imai, K. and Hashizume, K. (2004). Characterization of native and recombinant bovine pregnancy associated glycoproteins. *Res. Vet. Sci.*, **77**: 203"210.

Richardson, C., Herbert, C. N., and Terlecki, S. (1980). Determination of age of bovine fetus by crown-rump length. *Vet. Rec.*, **99**:22-24.

Roberts, R. M., Chen, Y., Ezashi, T. and Walker, A. M. (2008). Interferons and the maternal-conceptus dialog in mammals. Semin. Cell. *Dev. Biol* . 19(2):170-7.

Xie, S., Green, J., Beckers, J.F. and Roberts, R.M. (1995). The gene encoding bovine pregnancy-associated glycoprotein-1, an inactive member of the aspartic proteinase family. *Gene*, **159**:193-197.

FIG.1: AGAROSE GEL ELECTROPHORESIS OF PAG-1 GENE PCR PRODUCT FROM DIFFERENT DAYS OF GESTATION



LANE 1 to 7: 1181 bp PAG-1 gene PCR product obtained at different days of gestation (30, 50, 70, 90, 110, 150, full term, respectively)

LANE M : 100 bp DNA ladder as molecular size marker

ISSAR AWARDS

BEST CHAPTER AWARD

- The best chapter award will be given to a chapter based on its performance during the period between two successive conventions. The award will be in the form of trophy and a certificate of merit
- Eight copies of application for the best chapter award in the prescribed format should reach the General Secretary, ISSAR at least two weeks before the convention.
- Application form may be obtained from General Secretary, ISSAR.