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Expression of Placental Lactogen and Prolactein Related Protein-1 in the Placental Tissues during Gestation in Black Bengal Goat

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Abstract **– Placental lactogen, *PL* and Prolactein Related Protein-1, *PRP-1* are newly illustrated proteins synthesized from placenta in both bovine and caprine species having some major effect in gestation and fetal development. As Black Bengal goat (BBG) is the only recognized goat breed of Bangladesh, expression analysis of pregnancy specific genes in this breed is necessary to improve their reproductive efficiency. Aiming this fact, this study was designed to investigate the relative mRNA expression of this two genes (caprine Placental Lactogen, *cPL* and Prolactein Related Protein-1, *PRP-1*) in three different categories of caprine placental tissues (placentome, endometrium and fetal membrane) in three different stages of pregnancy (50, 60, 90 days) in Black Bengal goat. Same approach was performed in the same tissues using bovine placental lactogen, *bPL* which is a bovine specific protein. Analysis of relative mRNA expression of *cPL* and *cPRP-1* in different placental tissue samples indicates their strong expression in placentome and endometrium as early as at 50 days of gestational age with their expression at also 60 or 90 days. Whereas, *bPL* was not properly expressed in any placental tissues during 50, 60 or 90 days of gestation as *bPL* is pregnancy specific gene especially in bovine species. This expression analysis indicates that both *cPL* and *cPRP-1* has strong expression in Black Bengal does at as early as 50 days of gestation. So, *cPL* and *cPRP-1* may be used as candidate gene in pregnancy diagnosis in Black Bengal goat.**

Keywords **– Black Bengal Goat, Caprine Placental Lactogen, *cPL,* Caprine Prolactein Related Protein-1, *cPRP-1*, Endometrium, Fetal Membrane (FM), Placentome.**

I. INTRODUCTION

Placenta, a temporal organ plays a crucial role in pregnancy by producing a number of proteins during gestational period. Studies revealed that proteins like Placental Lactogen, *PL* and Prolactein Related Protein-1; *PRP-1,* both of which are member of prolactin gene family has a major effect in gestation which expressed early in the gestational period in both bovine and caprine species. Placental lactogen (*bPL*) and bovine prolactin-related protein-1 (*bPRP1*) are members of the growth hormone (GH)/prolactin (PRL) gene family, which are expressed simultaneously in Bovine trophoblast binucleate cells and are central to placentation and the progression of pregnancy in cattle [1]. Firstly the Placental Lactogen hormones of goats were detected by [2] which are polypeptide hormones produced by chorionic tissue in primates, rodents and ruminants [3, 4]. The *bPL* mRNA is transcribed in trophectoderm binucleate cells starting from Day 30 of pregnancy until the end of gestation which is involved in the regulation of ovarian function, mammogenesis, lactogenesis, and pregnancy stage-dependent adaptation of nutrient supplies to the fetus [5]. Placental lactogen (*PL*) [6, 7] specifically appear in both caprine (goat) and bovine trophoblast whereas in cattle prolactin-related protein (*PRP*) has been known as a placental-specific molecule that specifically expresses in trophoblastic binucleate cells. The production of *bPRP-I* during early implantation, and secretion into the uterine fluid and serum of pregnant cows [8], indicate that it may be a good candidate for pregnancy diagnosis [9].According to [10], Prolactin-related protein I (PRP-I) is a member of the nonclassical prolactin-related family and *PRP* genes in caprine placenta have coordination functions for gestation, as they do in bovine.

Pregnancy diagnosis and determination of litter size are of considerable value in improving efficiency of reproduction in goats. Accurate pregnancy diagnosis may provide essential information for effective herd management practices [11]. As Black Bengal goat is the most promising and valuable native goat breed of Bangladesh, improved reproductive managemental practices are major need for their economic production. In this concept, early pregnancy diagnosis is obviously one of the most important tools in this recognized native breed of Bangladesh.

Actually, pregnancy specific gene identification is of major need in small ruminant like goat. Expression analysis of these specific genes was considered to be essential to evaluate their relative expression in different placental tissues as there is very less information related to this specific gene expression in this goat breed.

The present study was, therefore, undertaken to determine the expression of this genes (placental lactogen and prolactin related protein) in placental tissues in

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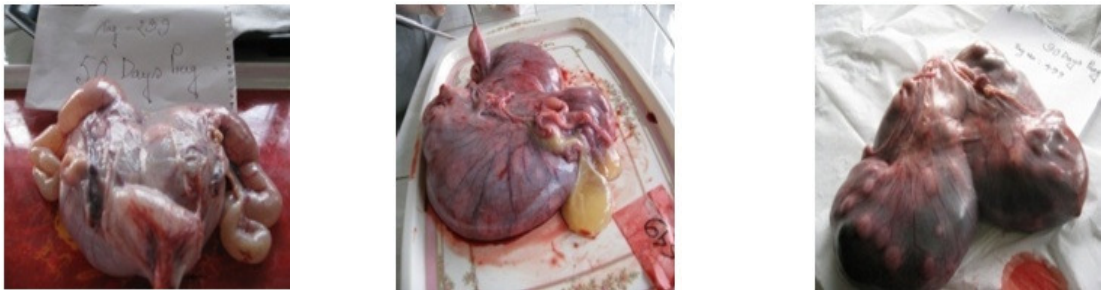
different days of pregnancy of Black Bengal goat to assist in molecular factor identification which will eventually be an aid for early pregnancy detection of this goat breed.

II. METHODOLOGY

Tissue samples were collected in Artificial Insemination Centre, Bangladesh Agricultural University, Mymensingh, Bangladesh and further study was conducted in reproductive Physiology Laboratory, Iwate University, Morioka, Japan.

Caprine uterine tissues intended for total RNA extraction, cDNA cloning and mRNA expression were collected from 3 pregnant Black Bengal does of three pregnancy stage (50, 60 and 90 days respectively).

The tissues collected across specific stages of gestation were designated as follows: (i) Day 50: placentomal, intracaruncular endometrium and fetal membrane tissue samples were collected from three different cows on days 50 of gestation (n = 3 animals) ; (ii) Day 60: placentomal, intracaruncular endometrium and fetal membrane tissue samples were collected from three different cows on days 60 of gestation (n = 3 animals) ; (iii) Day 90: : placentomal, intracaruncular endometrium and fetal membrane tissue samples were collected from three different cows on days 90 of gestation (n = 3 animals).After collecting these tissue samples they were immediately stored at RNA Later solution and preserved at 4°C. Pregnant placenta with fetus is shown in figure 1.



(a) (b) (c)

Fig.1. Pregnant placenta with fetus (a) 50 days pregnant placenta with fetus (b) 60 days pregnant placenta with fetus (c) 90 days pregnant placenta with fetus

After that, total RNA isolation from the respective tissue samples was done using RNeasy Minikit (Qiagen, Japan) according to manufacturer's protocol. Total RNA was quantified and its integrity was assessed using a spectrophotometer (by Nanodrop® ND-1000, Agilent's 2100 Bioanalyzer). Total RNA concentration was determined as the UV absorption 260/280 nm OD ratio with a Nanodrop ND-1000 spectrophotometer**.** Following that, reverse transcription (RT) of total RNA to single-stranded cDNA was done by using the High Capacity cDNA Reverse Transcription Kits (Applied Biosystem). PCR was performed using AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA). Caprine or bovine *GAPDH* was used as a positive control for the PCR. Bovine cDNA was used to see expression in bovine. The annealing temperature was primer specific, and the PCR involved 35 cycles. The PCR products were analyzed by agarose gel electrophoresis and visualized by ethidium bromide staining.

The respective primer sets for *bPL*, cPL, *cPRP1*, and *GAPDH* are listed in Table 1. PCR condition is listed in table 2 and 3.

Table 1: Primers used for RT-PCR

Genes	**Primer**	**Sequence**
GAPDH	Forward Reverse	CCTTCATTGACCTTCACTACATGGTCTA GCTGTAGCCAAATTCATTGTCGTACCA
bPL	Forward Reverse	CCATCTCCCCATCAGCAGCAGCAGT GAGACCCATTACACCCAAACAT
cPL	Forward Reverse	ATTCCCCTTCAAAGCCTGTT CACACTGGATAGGGCTCGTT
cPRP-1	Forward Reverse	CTATCAAGCGCCAGGAAGAT CTGGATGGGATGTCTGTGGG

Table 2: Thermal cycling conditions for RT-PCR

Stage	**Step**	**Temperature**	**Time**
Holding	Activation of AmpliTaq Gold® 360 Master Mix	95 °C	5 to 10 min‡
Cycling	Denature	95 °C	15 to 30 sec§
	Anneal	Primer Tm #	30 sec‡‡
	Extend	72 °C	60 sec/kb

Holding	Final Extension	72 °C	7 min
Holding	Final hold	4 °C	∞

Table 3: Annealing temperature and extension period for the target genes

Product	**Annealing temperature (°c)**	**Extension period (second)**
GAPDH	55	60
bPL	55	60
cPL	60	30
cPRP-1	60	30

III. Results and Discussion

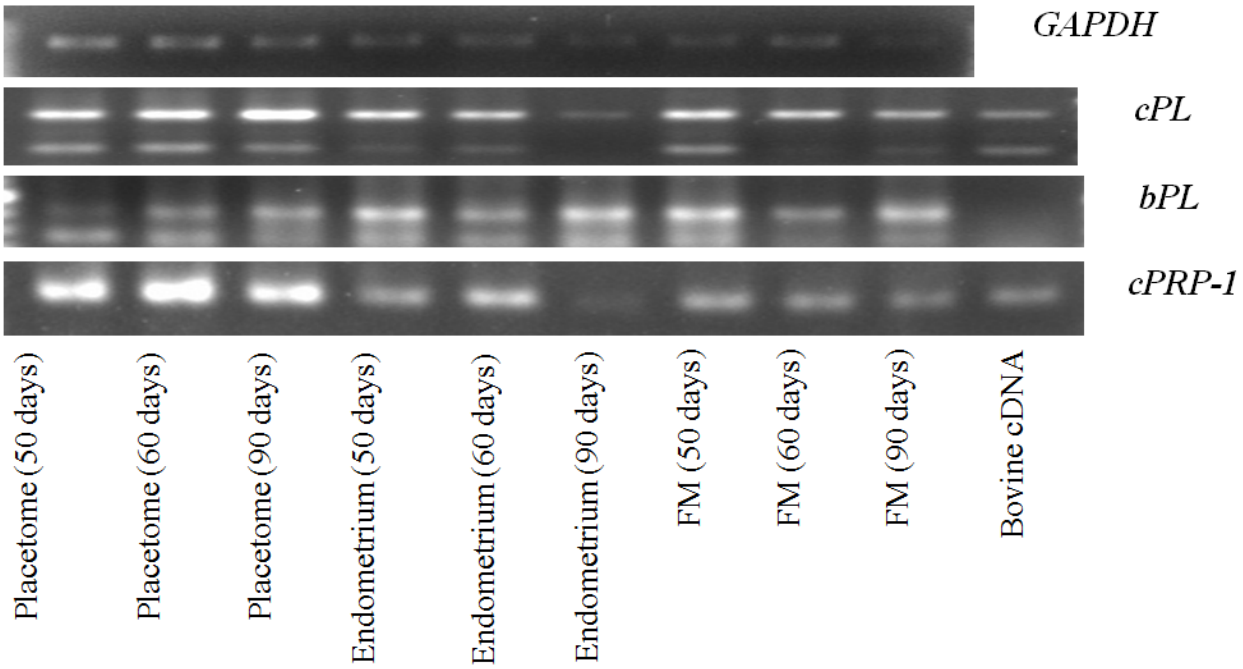


Fig.2. Relative mRNA Expression of *GAPDH, cPL, bPL, cPRP-1* for their gene specific primer in caprine placental tissues at three gestational stages.

Expression of GAPDH Gene around Day 50, 60 and 90 days of Gestation:

RT-PCR analysis confirmed GAPDH gene expression in Placental tissues in goat (Black Bengal goat). The band image (Figure2) shows strong single band for all the placental tissues; placentome, endometrium, fetal membrane (FT) of different gestational stage (50, 60 and 90 days respectively).

Expression of cPL Gene around Day 50, 60 and 90 days of Gestation:

RT-PCR analysis confirmed cPL gene expression in placental tissues of Black Bengal goat. Previously Caprine placental lactogen (cPL) cDNA was cloned by reverse transcription (RT)-PCR from total RNA of goat placenta [6]. The band image (Figure 2) shows double band for all the placental tissues containing a strong and a weak band. This represents that there may have some related gene expression during gestation in Black Bengal goat. Endometrial tissue around 90 days of gestation showed weak band, which may be the result of using low concentration RNA quality. The expression of this gene was less in fetal membrane during all days of gestation. . This result may indicate that, caprine Placental lactogen expresses as early as 50 days of gestation in Black Bengal goat as there was a strong band shown in the band image. Consequently plasma concentration of *cPL* may be high at 50 days of gestation in placentomes which may assist in pregnancy diagnosis in Black Bengal goat by possible radioimmuniassay. Along with that, there is a good scope to measure the expression of other related gene of Placental lactogen gene family in this goat breed.

Expression of bPL Gene around Day 50, 60 and 90 days of Gestation:

bPL is a bovine specific gene expressed during gestation. According to [9], the bPL mRNA is transcribed in trophoblast binucleate cells in bovine species. In the maternal circulation, *bPL* is detected in peripheral plasma following day 60 of gestation [12].While using its primer at caprine tissue DNA; it showed a relatively weak double band. This may indicate its less expression or expression of related gene of same gene family in caprine tissues. This result support the finding of [5] which states that the molecular structure and plasma profiles of bovine placental lactogen are very distinct from those described in

the sheep and goat. All the tissue samples of different stages of gestation exhibit the same pattern at their expression in band image (Figure 2) for *bPL*. This may indicate that, bovine Placental lactogen expression pattern may have some dissimilarity as in caprine species.

Expression of cPRP-1 Gene around Day 50, 60 and 90 days of Gestation:

RT-PCR analysis confirmed *cPRP-1* gene expression in placental tissues in goat (Black Bengal goat). The band image (Figure 2) shows strong single band for all the placentomal tissues of different gestational stage (50, 60 and 90 days respectively). According to [10] quantitative real-time RT-PCR analysis showed that the *cPRP1* expression intensities increased from Day 30 to Day 50 and then remained constant to Day 90 and thereafter decreased until Day 140 in placentome which is similar to present study. Similar result was found with bPRP-1 in bovine placental tissues in gestation in the study of [9], which stated that bovine prolactin-related protein I (*bPRP-I*) is the only protein in the prolactin family other than bovine placental lactogen (*bPL*) that has been shown to be expressed in the bovine placenta.

On the other hand, band image shows relatively weak band for endometrium and fetal membrane indicating their relative low expression at different stage of pregnancy (50, 60 and 90 days) in comparison to placentome. The similar result was found in the findings of [10] which states that in the caprine intercotyledon (the area between the cotyledonary villous and endometrium, the *cPRP1* expression intensity held steady from Day 30 to Day 90 and thereafter decreased until Day 140, although the intensity was low compared to that in placentome. This result indicates that, Prolactin Related Protein-1 expresses as early as 50 days of gestation in Black Bengal goat. Consequently plasma concentration of PRP-1 may be high at 50 days of gestation in placentomes which may assist in pregnancy diagnosis in BBG by possible radioimmuniassay.

IV. Conclusion

The RT-PCR analysis revealed that, *cPL* and cPRP-1was highly expressed in placentomes at all three stages of pregnancy, while less expressed in endometrium and fetal membrane which may be an indicative of some other related gene of *cPL* expression in the uterine wall and fetal membrane in the pregnant Black Bengal does. On the other hand, *bPL* gene was expressed at very low level and band image indicated expression of some other related gene in case of goat as *bPL* is a bovine specific gene identified earlier.

So it can be reported that caprine placenta may have the expression of this two genes at minimum 50 days of their gestational age. As a result, *cPL* and *cPRP-1* can be identified as candidate gene for early pregnancy determination in Black Bengal goat.

It may be noticed that this is the first study of gene expression analysis in this goat breed in Bangladesh. So, further studies are appreciated to identify more candidate genes and molecular markers for pregnancy detection with a view to establish an improved method of early pregnancy diagnosis in Black Bengal goat.

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