

Gastrin-Releasing Peptide (GRP) in the Uteroplacenta of the Sika Deer (*Cervus nippon*)

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Abstract: The localization of GRP in the uterus and/or placenta of non-pregnant or pregnant Sika deer was studied immunohistochemically. The strong immunoreactions were identified in the supra-nuclear region of the epithelial cells of the uterine glands of the placenta, however, no reaction was observed in the uterus of non-pregnant or immature animals. GRP may play an important role as the critical signals or nutrients for the growth of fetus in the synepitheliochorial placenta.

Key words: Immunolocalization, GRP, Sika deer, uterus, placenta, Japan

INTRODUCTION

The study of the Sika deer has been done by several points of view including the reproductive status (Suzuki and Ohtaishi, 1993), the testicular and ovarian histology (Hayakawa *et al.*, 2004), the anatomical study (Ozaki *et al.*, 2004), the endocrine study (Takahashi *et al.*, 2002) and phylogenetic study (Nagata *et al.*, 1999). The necessity of the reproductive study of Sika deer can be emphasized for the accumulation of the basic reproductive information for the purpose of the artificial control of the over populated Sika deer in Japan. The emphasize will be also pointed for the conservation and artificial production of endangered Sika species in several countries including China and Vietnam.

The Gastrin-Releasing Peptide (GRP) has been know to regulates numerous central nervous and gastrointestinal functions (Lesniewska *et al.*, 2001). The important role of GRP including the modulation of uterine contraction (Amiot *et al.*, 1993), the control of epithelial ion transport in the endometrium (Matthews *et al.*, 1993) and an autocrine and paracrine regulation in the uterus (Vetter and O'Grady, 1997) has been suggested. The GRP immunolocalization has been studied in human (Xiao *et al.*, 1996), sheep (Fraser *et al.*, 2004), cattle (Budipitojo *et al.*, 2001) and mouse deer (Kimura *et al.*, 2006) and the localization in the uterine epithelium were demonstrated. However, no studies has been done in any cervid species.

This study was designed to clarify the physiological role of the uteroplacenta of Sika deer by using a GRP as

an indicator to compare with other ruminants. Such information is a necessary prerequisite for design of effective strategies for the management and conservation of this species.

MATERIALS AND METHODS

Totally 28 female Sika deer were used in this study. Sika deer were killed by the rifle or shotgun shots for the culling for the purpose of the population control or the pleasure hunting in the Tanzawa-Oyama Kanagawa Prefectural Natural Park, Japan during November to March of 2004-2006. Among the twenty eight deer, the existence of the fetus were macroscopically recognized in three individuals and the Hind Foot Length (HFL) of the fetus were 16.5 mm in No.: 804045, 97.5 mm in No.: 804046 and 119.5 mm in No.: 804020. Uteri were excised and fixed in 10% formalin or Bouin's solution. They were dissected and samples were dehydrated, embedded in paraffin and stained immunohistochemically using the avidin-biotin-peroxidase complex method (Hsu *et al.*, 1981) with the antisera to porcine GRP raised in the rabbit (Yanaihara Institute Inc., Shizuoka, Japan, Code Y160). Briefly, after an overnight incubation at 4°C with the primary antiserum (x10,000 dilution), sections were incubated with biotinylated anti rabbit immunoglobulins (Nichirei, Tokyo, Japan) for 30 min. Following this step, the avidin-biotin-peroxidase complex was applied to the sections for 5 min. A brown reaction product was obtained by subsequently incubating the sections in diaminobenzidine-H₂O₂ solution. Staining controls included the omission of

the primary antiserum. All stained sections were observed by light microscope attached with a digital camera (Coolscope, Nikon, Tokyo, Japan).

RESULTS AND DISCUSSION

Binucleated cells which is common and unique for ruminant placenta were identified at the trophoblast (Chorionic epithelium) of placenta in Sika deer as reported in other deer species like Roe deer, Fallow deer, Chinese water deer, Muntjac deer, Red deer and Axis deer (Wooding, 1982). From this finding, the placenta of Sika deer can be classified as a synepitheliochorial placenta, a mixture of maternofetal hybrid synepithelium with cellular uterine epithelium apposed to fetal cellular trophoctoderm.

The crossreaction of antisera to porcine GRP with the uteroplacental tissue of Sika deer was verified. In the uteri of the immature (No.: 804023, Fig. 1) and non pregnant (No.: 804022, Fig. 2) Sika deer, the immunoreaction for the GRP was not detected. On the other hand, strong positive reactions were detected in the uterine glands of the all pregnant Sika deer (No.: 804045, Fig. 3a and b, No.: 804020, Fig. 3c and d).

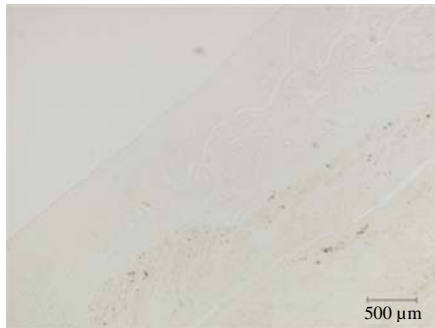


Fig. 1: Negative GRP immunoreaction in the endometrial glands of immature Sika deer (No.: 804023)

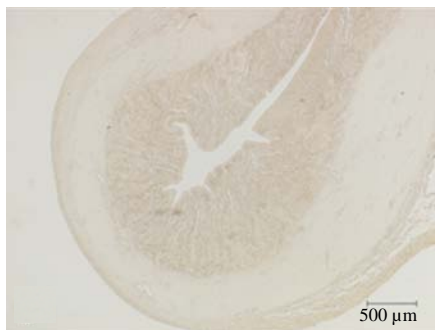


Fig. 2: Very weak or negative immunoreaction of GRP in the endometrial glands of the non-pregnant mature Sika deer (No.: 804022)

The supra nuclear region of the uterine epithelial glandular epithelial cells show vividly positive reaction for the GRP antibody (Fig. 3b and d). Uterine epithelial cells, stromal and muscular layer did not show positive reactions. The localization of GRP in the supra nuclear region of the uterine glands suggests that GRP was released from the cells into the lumen of the gland by exocrine manner. This was also evident in the cow at light and electron-microscopic levels (Budipitojo *et al.*, 2001, 2003) and in the mouse deer at light-microscopic level (Kimura *et al.*, 2006).

The immunoreactivity of GRP in the endometrial glands was much stronger in the pregnant individuals, regardless of the size of fetus (HFL 16.5-119.5 mm). On the other hand in the uterus where no fetus was observed macroscopically, the GRP immunoreactivity was negative (Fig. 1 and 2).

GRP immunoreactivity in the reproductive system has been detected during human pregnancy, predominantly in the trophoblasts of placental villi, extravillous trophoblasts and amniotic epithelial cells (Xiao *et al.*, 1996). In sheep and cattle, it has been reported both in non-pregnant and pregnant animals, predominantly in the uterine gland epithelial cells which show immunolocalization by this peptide (Budipitojo *et al.*, 2001).

This study is the first to show the immunoreaction of GRP in the animals belonging to Cervidae family. This result indicate that the GRP plays an important role as the critical signals or nutrients for the growth of fetus especially in the pregnant period. In previous results, the GRP is identified positively in the pregnant uterus and weakly or negatively in the non pregnant uterus in the mouse deer (Kimura *et al.*, 2006). Although, there is the

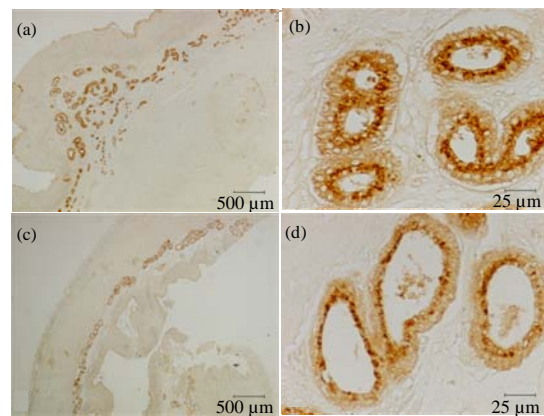


Fig. 3: a-d) Strong positive immunoreaction of GRP in the supra nuclear region of the endometrial glandular epithelial cells of the pregnant Sika deer (a, b, No.: 804045 and c, d, No.: 804020)

differences in the level of suborder between Sika deer (Pecora) and Mouse deer (Tragulina), Sika deer as the seasonal breeder is showing the much clearer differences of the immunohistochemical reactivity between pregnant and non pregnant stage compared to the mouse deer as non seasonal breeder.

This speculation can be adopted to the difference between Sika deer and cow which both are belonging to suborder Pecora. In the cow the weakly positive reaction of GRP can be observed even in the non pregnant uterus (Budipitojo *et al.*, 2003). It was revealed in the cow that RT-PCR showed GRP mRNA fragments in the tissue during all stages of the cycle (Budipitojo *et al.*, 2003). Positive staining of GRP immunoreactivity in the uterine gland epithelial cells was also detected in both the uterine horn and body during all stages of the cycle in the cow (Budipitojo *et al.*, 2003). Sika deer delivers only one time in the year in Japan. On the other hand, cow in the captivity in Japan possesses multiple breeding season in a year. The difference of the existence of the breeding seasonality may cause the clear difference in the expression the GRP in the uterus.

CONCLUSION

The strong immunoreactions were identified in the epithelial cells of the uterine glands of the placenta in the Sika deer. GRP may play an important role as the critical signals or nutrients for the growth of fetus in the synepitheliochorial placenta.

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