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Immunohistochemical localization of kisspeptin and its receptor in the placentome of buffalo (*Bubalus bubalis*)

G K MISHRA[1], M K PATRA[2]✉, M KARIKALAN[2], S K SINGH[2], H KUMAR[2] and N KRISHNASWAMY[3]

ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

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Kisspeptinergic system, composed of kisspeptin (Kp) and its cognate G-protein coupled receptor (Kiss1r), stimulates GnRH pulses and is essential for the onset of puberty, transition from non-breeding to breeding season and ovulation in many mammals (Caraty *et al*. 2011). Immunolocalization of Kp and its receptor has been demonstrated in the hypothalamus and ovary in the cyclic and acyclic buffalo (Mishra *et al.* 2019 a, b and c). Apart from the hypothalamic and extrahypothalamic sites, trophoblastic cells of human and rat placenta show intense immunoreactivity (Terao *et al*. 2004, Hu *et al*. 2019). High degree of expression and dramatic increase of Kp in the human placenta during the first trimester of pregnancy (Horikoshi *et al*. 2003) indicates a possible role in the luteal development and progesterone secretion similar to human chorionic gonadotrophin hormone (Niswender *et al*. 2000). Recently, a preliminary study demonstrated the circulating Kp in the pregnant cows (Mondal *et al.* 2015) and even in early post-partum dairy cows (Rizzo *et al*. 2019). To the best of our knowledge, localization of Kp and its receptor, Kiss1r in the placentome of ruminants is not known. The present study demonstrates the immunohistochemical evidence of Kp and Kiss1r in the bubaline placentomes.

Pregnant uteri were collected from a local buffalo slaughter house, in Bareilly, Uttar Pradesh and brought to the laboratory on ice. The gravid horn was excised and the fetus along with the fetal membranes was examined. The gestational age of the fetus was estimated from the crown rump length following the standard formula validated for buffalo. The fetal age was approximately 120 days. The dome shaped placentome comprising of caruncle and fetal cotyledon was collected from the middle region of the greater uterine curvature (Fig. 1 A-B) and was preserved in 10% neutral buffered formal saline for histology and immunohistochemistry. The preserved placental tissue samples were trimmed and processed overnight and washed under tap water, then dehydration of samples was done through ascending grades of alcohol, followed by clearing with acetone and benzene. The tissues were embedded in paraffin and serial sections of 4-5 µm thickness were cut through microtome. Finally, tissue sections were stained with haematoxylin and eosin and observed under light microscope.

The serial sections of placental tissue were mounted

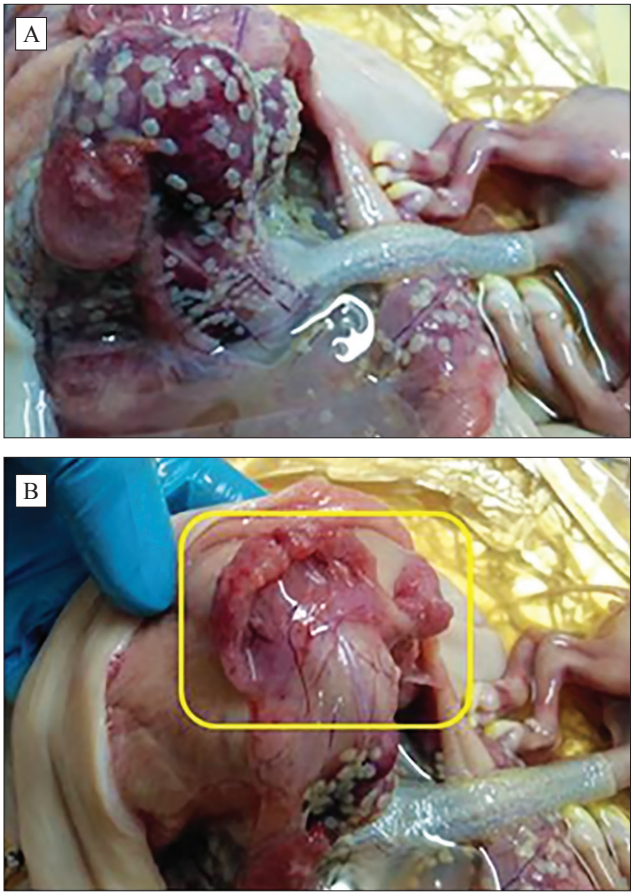

Fig. 1A-B. Representative gross image of a placentome (yellow square box) from a buffalo with a gestation age of approximately 120 days.

Present address: [1]Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Kuthulia, Rewa, Madhya Pradesh. [2]ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh. [3]ICAR-Indian Veterinary Research Institute, Bengaluru campus, Hebbal, Bengaluru, Karnataka. ✉Corresponding author email: drmanas01@gmail.com

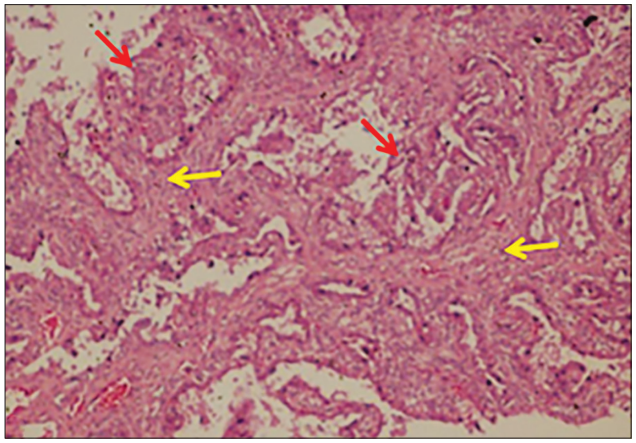

Fig. 2. Representative photomicrograph of H&E stained sections showing the cross section of placentome. Fetomaternal interface can be appreciated by the closely apposed caruncles (yellow arrows) and cotyledons (red arrows).

on 3-aminopropyltriethoxysilane (2% APES) coated glass slides and dried overnight at 37°C. The sections were deparaffinized in xylene; rehydrated in a series of graded alcohol at room temperature, subjected to antigen retrieval using proteinase K for 20 min at 37°C, rinsed and blocked with 5% kid serum for 1 h at room temperature. Subsequently, the sections were incubated with the anti Kiss1 (0.2 mg/mL; 1:100 dilution, GeneTex, USA) and anti Kiss1r (1 mg/mL; 1:50 dilution, GeneTex, USA) rabbit polyclonal primary antibody at 4°C for 16 h. After three washes of 5 min each, goat anti-rabbit IgG-HRP (GeNei Laboratories Pvt. Ltd., Bengaluru) was added as secondary antibody and incubated for 2 h. Tissue sections were incubated for 3 min in freshly prepared peroxidase solution (10 mg DAB, Sigma-Aldrich, USA), dissolved in 10 mL 50 mM sodium phosphate buffer, *p*H 7.4 in which 1.25 µL hydrogen peroxide was added. Positive immunoreaction was indicated by the development of brown colour in the tissue sections and finally the slides were rinsed three times for 5 min with distilled water. In the negative control, primary antibody was omitted. All the sections were counter-stained with Mayer's haematoxylin, mounted with DPX and examined under microscope by a veterinary pathologist.

Two morphologically distinct cell types consisting of mononucleate trophoblast cells and binucleate trophoblast giant cells (BNC) were observed at the feto-maternal interface of placentome. Several clusters of BNC were interposed between the mononucleate trophoblast cells forming typical characteristic of ruminant placenta (Fig. 2). Intense Kp immunoreactivity was observed in the BNC at the fetomaternal lining (Fig. 3A-B). The expression of Kiss1r was also moderate to intense (Fig. 3C-D). The predominant expression of Kp in the BNC suggests a role in the placental function. Our findings are in accordance with the reports in the human placenta, where the high concentration of Kp was observed in the plasma throughout gestation although its production was detected from the early pregnancy (Horikoshi *et al*. 2003). This observation

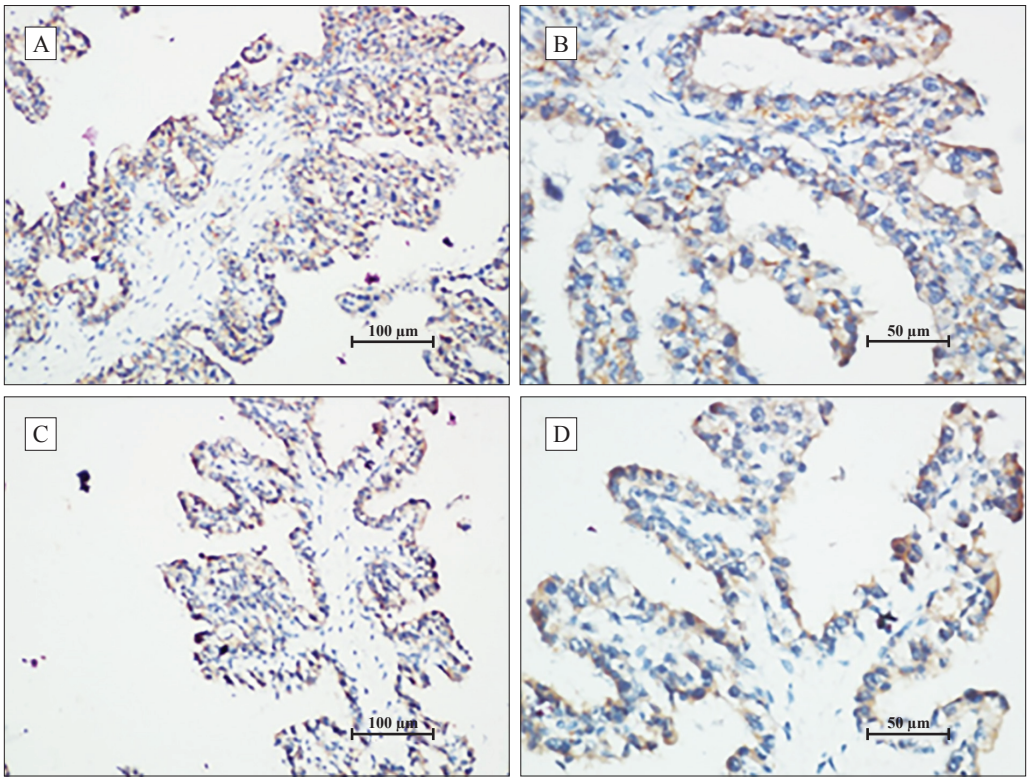

Fig. 3. Representative photomicrographs showing immunohistochemical localization of Kisspeptin (Kp) and Kisspeptin receptor (Kiss1r) in the placentome of pregnant buffalo. A-B. Immunolocalization of Kp is indicated by the appearance of dark brown colour staining cells along the lining of cotyledons. C-D. Note the moderate to intense expression of Kiss1r in the binucleated cells lining the cotyledons; Bar: 100 µm and 50 µm.

suggests a physiological role of Kp from implantation to delivery in human reproduction. The expression of Kiss1r was also demonstrated in the placental tissue using qPCR and the ligand protein was purified from the human placenta (Kotani *et al*. 2001). In the placenta, the Kiss1 gene transcript was significantly upregulated as compared to other extrahypothalamic sites such as pancreas, liver and small intestine. Similarly, Kiss1r gene transcripts were upregulated in the placenta and pancreas relative to the moderate expression in the spleen, peripheral blood leukocytes, testis and lymph node in the human (Ohtaki *et al*. 2001). It is concluded that BNC of the placentomes from the pregnant buffaloes of 90 to 120 days of gestation strongly express Kp and its receptor, suggesting possible role of Kp in the regulation of placental function in ruminants.

SUMMARY

Evidence for extra-hypothalamic expression of kisspeptinergic system is emerging. The present study documents the imuunolocalization of the kisspeptin and its receptor in the placentome of buffalo. Gravid uterus was collected from the abattoir and gestational age was estimated by crown-rump length of the fetus. A section of placentome containing the caruncle and cotyledon was used for immunohistochemistry. The expression of Kp and its receptor was evident in the feto-maternal junctions with prominent localization in the mononucleate and binucleate cells. In conclusion, the present study supports possible role of placental kisspeptin during pregnancy in the ruminants.

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REFERENCES

Caraty A, Franceschini I and Hoffman G E. 2011. Kisspeptin and the preovulatory gonadotrophin-releasing hormone/ luteinising hormone surge in the ewe: Basic aspects and potential applications in the control of ovulation. *Journal of Neuroendocrinology* **22**: 710–15.

Horikoshi Y, Matsumoto H, Takatsu Y, Ohtaki T, Kitada C, Usuki S and Fujino M. 2003. Dramatic elevation of plasma metastin concentrations in human pregnancy: Metastin as a novel placenta-derived hormone in humans. *Journal of Clinical Endocrinology and Metabolism* **88**: 914–19.

Hu K L, Chang H M, Zhao H C, Yu Y, Li R and Qiao J. 2019. Potential roles for the kisspeptin/kisspeptin receptor system in implantation and placentation. *Human Reproduction Update* **25**(3): 326–43.

Kotani M, Detheux M, Vandenbogaerde A, Communi D, Vanderwinden JM, Le Poul E, Brezillon S, Tyldesley R, Suarez-Huerta N, Vandeput F, Blanpain C, Schiffmann S N, Vassart G and Parmentier M. 2001. The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein coupled receptor GPR54. *The Journal of Biological Chemistry* **276**: 34631–36.

Mishra G K, Patra M K, Singh L K, Sheikh P A, Upmanyu V, Chakravarti S, Karikalan M, Sonwane A, Singh S K, Das G K, Kumar H and Narayanan K. 2019b. Expression of kisspeptin and its receptor in the hypothalamus of cyclic and acyclic buffalo (*Bubalus bubalis*). *Theriogenology* **139**: 167–77.

Mishra G K, Patra M K, Singh L K, Upmanyu V, Chakravarti S, Karikalan M, Singh S K, Das G K, Kumar H and Narayanan K. 2019a. Kiss1 and its receptor: Molecular characterization and immunolocalization in the hypothalamus and corpus luteum of the buffalo. *Animal Biotechnology* **30**(4): 342–51.

Mishra G K, Patra M K, Singh L K, Upmanyu V, Chakravarti S, Karikalan M, Bag S, Singh S K, Das G K, Kumar H and Narayanan K. 2019c. Expression and functional role of kisspeptin and its receptor in the cyclic corpus luteum of buffalo (*Bubalus bubalis*). *Theriogenology* **130**: 71–8.

Mondal M, Baruah K K and Prakash B S. 2015.Determination of plasma kisspeptin concentrations during reproductive cycle and different phases of pregnancy in crossbred cows using bovine specific enzyme immunoassay. *General and Comparative Endocrinology* **224**: 168–75.

Niswender G D, Juengel J L, Silva P J, Rollyson M K and McIntush E W. 2000. Mechanisms controlling the function and life span of the corpus luteum. *Physiological Reviews* **80**: 1–29.

Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kanehashi K, Terao Y, Kumano S, Takatsu Y, Masuda Y, Ishibashi Y, Watanabe T, Asada M, Yamada T, Suenaga M, Kitada C, Usuki S, Kurokawa T, Onda H, Nishimura O and Fujino M. 2001. Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature* **411**: 613–17.

Rizzo A, Ceci E, Guaricci A C and Sciorsci R L. 2019. Kisspeptin in the early post–partum of the dairy cow. *Reproduction in Domestic Animals* **54**(2): 195–98.

Terao Y, Kumano S, Takatsu Y, Hattori M, Nishimura A, Ohtaki T and Shintani Y. 2004. Expression of KiSS-1, a metastasis suppressor gene, in trophoblast giant cells of the rat placenta. *Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression* **1678**: 102–10.