Annexure VIII

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

Final Report of the work done on the Major Research Project

1. **Project report No**. Final
2. **UGC Reference No.** : F.No. 41-32/2012 (SR)
3. **Period of report**: from 01.07.2012 to 30.06.2105

4. **Title of research project** "Immunohistochemical localization of estrogen and progesterone receptors in female genitalia of buffaloes"

5. **(a) Name of the Principal Investigator**: Dr. Devendra Pathak
   (b) Deptt. Veterinary Anatomy
   (c) University/College where work has progressed: College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana

6. **Effective date of starting of the project:** 01.07.2012

7. **Grant approved and expenditure incurred during the period of the report:**
   a. Total amount approved Rs. 8,22,500
   b. Total expenditure Rs. 8,14,884

Audited statement of account (**Annexure III**), statement of expenditure incurred on field work (**Annexure IV**), Audit utilization certificate (**Annexure V**), information with respect to staff appointed (**Annexure VI**) and house rent of project fellow (**Annexure VII**) are attached.

c. Report of the work done: (**Enclosure I attached**)

Annexure VIII

i. Brief objective of the project:

1) To characterize the distribution of estrogen and progesterone receptors in ovaries of buffaloes during follicular and luteal phases of estrous cycle
2) To characterize the distribution of estrogen and progesterone receptors in oviduct uterus and vagina of buffaloes during follicular and luteal phases of estrous cycle.
3) To characterize the distribution of estrogen and progesterone receptors in ovary, oviduct, uterus and vagina of buffaloes during different seasons.
4) To study the blood hormone level of estrogen and progesterone with respect to localization of receptors during follicular and luteal phase and during different seasons.

ii. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication.

Papers published/Submitted:

1. Pathak D, Bansal N, Singh O, Gupta K and Ghuman SPS. 2018. Immunohistochemical localization of Estrogen receptor alpha (ERα) in oviduct of buffalo during follicular and luteal phase of estrous cycle. *Tropical Animal Health and Production. TROP-D-18-01245 (In review).*

2. Pathak D, Bansal N, Singh O, Gupta K and Ghuman SPS. 2018. Immunolocalization of Progesterone Receptor (PR) in Oviduct of Indian Buffalo during Follicular and Luteal Phases of Estrous Cycle. *Journal of Animal Research (In review)*

3. Pathak D, Bansal N, Singh O, Gupta K and Ghuman SPS. 2018. Immuno Localization of Estrogen Receptor (ERα) and Progesterone Receptor (PR) in Uterus of Buffalo during Follicular and Luteal Phases of Estrous Cycle. *Turkish Journal of Veterinary and Animal Sciences. (In review).*

Papers Presented and Abstracts Published (Enclosure IIa attached)

1. Pathak D, Bansal N, Gupta K and Ghuman SPS. 2012. "Immuno localization of Estrogen receptor in oviduct of buffalo during follicular and luteal phase of estrous cycle" at XXVIIth Annual Convention of Indian Association of Veterinary Anatomists and National Symposium at Department of Veterinary Anatomy and Histology, KAVASU, Thrissur from 28-30, November, 2012.

2. Pathak D, Bansal N, Gupta K and Ghuman SPS. 2014. "Immunolocalization of progesterone receptor in oviduct of buffalo" *at XXVIIIth Annual Convention of Indian Association of Veterinary Anatomists and National Symposium* at Department of Veterinary Anatomy, Rajasthan University of Veterinary And Animal Sciences, Bikaner during 8-10, January, 2014.

3. Pathak D, Bansal N and Gupta K. 2014. "Immunolocalization of estrogen receptor alpha in uterus of buffalo" at *XXVIIIth Annual Convention of Indian Association of Veterinary Anatomists and National Symposium* at Department of Veterinary Anatomy, Rajasthan University of Veterinary And Animal Sciences, Bikaner during 8-10, January, 2014.

4. Pathak D, Bansal N and Gupta K. 2014. "Immunolocalization of estrogen receptor alpha and progesterone receptor in cervix uteri of Indian buffalo" at *International conference on "Reproductive Health: Issues and stretegies under changing climate scenario*" and 24th Annual meet of ISSRF held from 6th – 8th February, 2014 at IVRI, Izatnagar.

5. Pathak D and Bansal N. 2016. "Histomorphochemical features and progesterone receptor expression in atretic follicles in Indian buffalo" Dr. S.S. Guraya Memorial Seminar of Advances in Animal Reproduction held at Dept of Zoology, PAU Ludhiana 14100

Awards for Papers Presentations (Enclosure IIb attached)

1. **Best Paper presentation Award and Medal 2012** for "Immuno localization of Estrogen receptor in oviduct of buffalo during follicular and luteal phase of estrous cycle" in Histoenzymology and Immunohistochemistry.

2. **Best Poster presentation Award and Medal 2014** for "Immunolocalization of progesterone receptor in oviduct of buffalo".

3. **Best Paper presentation Award 2014 for** "Immunolocalization of estrogen receptor alpha in uterus of buffalo" in Histoenzymology and Immunohistochemistry".

iii**. Has the progress been according to original plan of work and towards achieving the objective**? If not, state reasons: **Yes**, Progress has been according to the plan of the work towards achieving the objectives.

iv. **Please indicate the difficulties, if any, experienced in implementing the project____**NA---

v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet. ---NA---

Annexure VIII

vi. If the project has been completed, please enclose a summary of the findings of the study. One bound copy of the final report of work done may also be sent to University Grants Commission.

vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph. D. awarded (c) Publication of results (d) other impact, if any. –NA-

Signature of the
Principal investigator

Director of Research
(Seal)

Signature of the
Co-investigator

Signature of the
Head of the Department

Enclosure I (Report of the Work Done)

Introduction:

The buffalo forms the backbone of India's dairy industry and is rightly considered as the 'bearer cheque' of the rural flock considered as India's milking machine (Balain, 1999). The buffalo plays a very important role in Indian economy as it alone contributes about 56% of total milk production in India. The river buffaloes (*Bubalus bubalis*) of the Indian sub-continent are maintained chiefly for milk production, but all of them are also dual purpose animals, exhibiting good meat characteristics (Das *et* al 2008). The proposal originates from one of the most important and practical problem of low fertility in buffaloes.

The different actions of the steroid hormones along the reproductive tract are dependent on the presence of the hormone and its specific receptor concentration in the target tissue, which is also regulated by estrogen and progesterone (Clark *et al.* 1992). Estrogens and progesterone are the main hormones modulating the function of the female reproductive tract by operating through specific intracellular receptors (ER and PR, respectively).

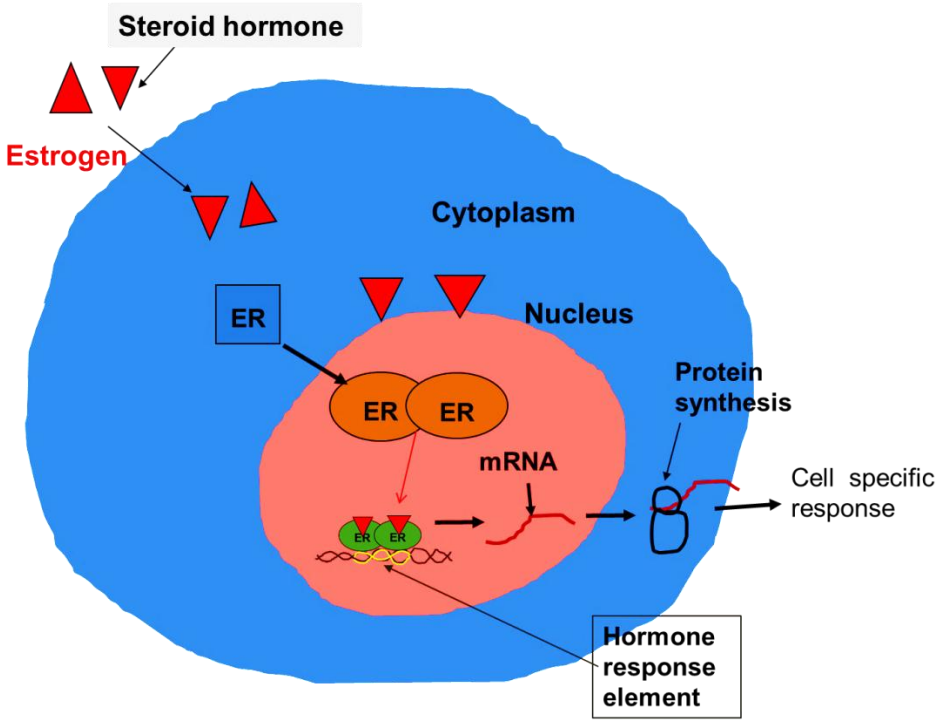


Fig. A. Mechanism of Steroid Hormone action

Mechanism of Steroid Hormone action (Fig. A): The steroid hormones are secreted as prohormones and as they reach to the target sites get converted into hormones which then bind to the receptors which leads to the dimerization of hormone which in turn initiate binding to hormone response element (HRE). It leads to the transcription of mRNA and thus desired proteins for its action. Thus presence of their receptor is mandatory for their actions.

The importance of estrogens for normal growth and differentiation of the reproductive tract has been reported (Greco *et al.* 1993, Gorski and Hou 1995). Estrogen Receptors (ERs) mediate the important actions of the endogenous steroid hormone, 17β-estradiol (E2), and thereby participate in various aspects of cellular physiology. The estrogen signaling system plays crucial role in the development of normal functions of female reproductive tract, secondary sex characteristics and in reproductive behavior. A recent study has described the uterine changes in ER and PR expression during the early postnatal period in the lamb and the results support the hypothesis that ERα is also necessary for normal uterine growth and development in ovine (Taylor *et al.* 2000).

Progesterone mediates various effects in the female reproductive organs through its cognate nuclear receptors, PR-A and PR-B, which often are co-expressed within the same cells, e.g., in granulosa cells of preovulatory follicles (Gava *et al.*, 2004). The progesterone receptor (PR) is an estrogen-regulated protein and proposed that expression of PR determination indicates a responsive estrogen receptor (ER) pathway to predict response to endocrine therapy in mammary tumour. The earlier studies showed that PR determination provides supplementary information to ER, both in predicting response to endocrine therapy and estimating survival. PR has proved superior to ER as a prognostic indicator in some studies.

The present work was designed to study the immuno localization of estrogen and progesterone receptors in female genitalia of buffaloes.

Review of literature

The cellular distribution of estrogen receptor has been characterized with autoradiography using radioactive estrogens, initially in rat (Stumpf *et al.*, 1971) and subsequently in human (Tchernitchin *et al.*, 1973) reproductive tissues. These studies showed that ^{3}H-estradiol was localized over the nuclei of epithelial cells, of underlying connective tissues (substantia propria), and of muscularis in the vagina, uterus, and oviduct of rat and human.

Miller *et al.* (1977) have found high rates of protein synthesis and increased RNA: DNA ratios in the ovine endometrium and oviduct at or shortly after estrus, associated with an increased E2 level in plasma. Progesterone action on RNA and protein synthesis differs in the ovine endometrium and isthmic oviduct, with highly significant increases in the former but no effect in the latter (Meikle *et al.* 1997), since the administration of P4 increased uterine weight but had no effect on the oviductal and cervical weights, differing from E2 treatment after which all parts of the reproductive tract increased in weight (Meikle *et al.*, 1997).

ERs act primarily as nuclear transcription factors, and this effect is enhanced by ligand binding (Levin, 2001). ER-α was predominant in the oviduct, uterus and cervix of the rat (Wang *et al.*, 1999). Presently, two estrogen receptors (ERs), ER-α and ER-β, have been cloned in mammals, and they are expressed in many cell types of metazoans.

High levels of ER and PR in cervix and oviduct were found in the female lamb, differing from other mammalian species. No significant effects by either E2 or P4 treatment on ER and PR levels in the cervix and oviduct could be observed. E2 treatment increased the mRNA levels of ERα and PR more than 3-fold in the cervix, while P4 treatment increased the mRNA levels of ERα and PR in the uterus. The results show differential effects of gonadal steroids on sex steroid receptor expression along the reproductive tract in female lambs, suggesting that steroid target tissues can modulate responses to the same circulating levels of steroid hormones (Meikle *et al.*, 2001).

Okada *et al.* (2003) studied the expression of estrogen, and progesterone receptors in the oviduct of developing, cycling and pre-implantation rats. He observed all receptors except for ERβ in epithelial and stromal or mesenchymal cells of the fetal and neonatal oviduct. During the estrous cycle and early pregnancy, ERα and PR-A+B were expressed in epithelial, stromal and muscle cells throughout the oviduct region, and showed changes in expression predominantly in the isthmus. Only a few epithelial cells in the infundibulum and ampulla showed ER-β staining.

PR was localized predominantly in the nuclei of epithelial, stromal, and muscle cells in the uterus and vagina during the estrous cycle. In the uterus, the nuclei of epithelial cells were stained intensively at diestrus, while the PR staining of the stromal cells was more intense at proestrus than at any other stage of the cycle. PR expression during the cycle in muscle cells of the myometrium was similar to that in the endometrial stromal cells. In the vagina, however, PR expression during the cycle was approximately the same among epithelial, stromal, and muscle

cells, the nuclei of which were stained deeply at proestrus. Ovariectomy at various stages of the cycle altered the PR expression appearing in the uterus and vagina during the cycle (Ohta *et al.*, 1993).

Teilmann *et al.* (2006) studied the expression and localization of the progesterone receptor in mouse and human reproductive organs. They observed that in the oviduct ciliated epithelial cells of adult mice and human revealed a unique PR localization to the lower half of the motile cilia. PR immunolocalization to the oviduct cilia was greatly increased in pubertal mice upon hCG stimulation. They suggested that ciliary PR in the oviduct plays a role in progesterone signaling after ovulation, possibly via non-genomic events.

Methodology

A. Collection of samples

The tissue samples of ovary, oviduct and Uterus of buffalo were collected from the abattoirs in different seasons of a year. After checking the status of the ovaries the samples were classified into follicular and luteal phase of estrous cycle. Precise stages of estrous cycle were determined by histological observation of the ovary. After thoroughly washing in buffer, tissues were placed for fixation in buffered formalin for histomorphochemical and immuno-histochemistry and Karnovsky's fixative for electron microscopy and immuno-cytochemistry.

B. Processing of tissue samples for histology, histochemistry and histoenzymic studies:

After checking the status of the ovaries they were classified into follicular and luteal phase of estrous cycle. After thoroughly washing in buffer, tissues were placed for fixation in 10% buffered formalin for histomorphochemical studies using routine acetone benzene schedule and unfixed tissue were stored at -200C for histoenzymic studies.

C. Processing of tissue samples for immuno histochemistry:

After thoroughly washing in buffer, tissues were placed for fixation in buffered formalin and were processed by acetone benzene schedule. The paraffin embedded tissue sections were sectioned and placed on positively charged glass slides. These sections were stained with primary and secondary antibodies against estrogen and progesterone hormones to characterize estrogen and progesterone receptors using one step horseradish peroxidase method.

D. Processing of tissue samples for electron microscopy

The tissues were thoroughly washed in phosphate buffer saline solution and were fixed for 2 hour in Karnovsky's fixative, and then secondary fixation in 2% osmium tetra oxide for two hours Subsquently tissue samples were subjected to dehydration in ascending grades of acetone. After clearing and infiltration, tissues were embedded in pure embedding media using beam capsule and polymerization was undertaken. The prepared grids were stained with lead citrate and urinyl acetrate and viewed in TEM.

E. Processing of tissue samples for Immuno electron microscopy using immunogold technique

The grids prepared for ultrastructural study were incubated with primary and secondary antibodies against estrogen and progesterone hormones to characterize estrogen and progesterone receptors in the cells.

F. Estimation of estrogen and progesterone hormone in the blood samples:

Plasma Estrogen Analysis

Estradiol hormone in blood serum was estimated using ELISA kit for estradiol (LabServ, Thermo Fisher Scientific, Catalogue No. DKT003).

Plasma progesterone analysis:

Plasma progesterone was estimated by liquid phase Radioimmunoassay (RIA) procedure using progesterone antisera raised in the Department of Veterinary Gynaecology and Obstetrics, GADVASU, Ludhiana (Ghuman *et al.*, 2009).

Results:

Experiment 1. Standardization of Immunohistochemical protocol for localization of ER and PR

Protocol for immunohistochemical localization of estrogen and progesterone receptors was standardized. In the present study super sensitive one step polymer based horseradish peroxidase method was standardized for our laboratory conditions. The dilutions for the ER antibody were 1:500 (Monoclonal antibody) and for the PR antibody was 1:5000 (polyclonal antibody).

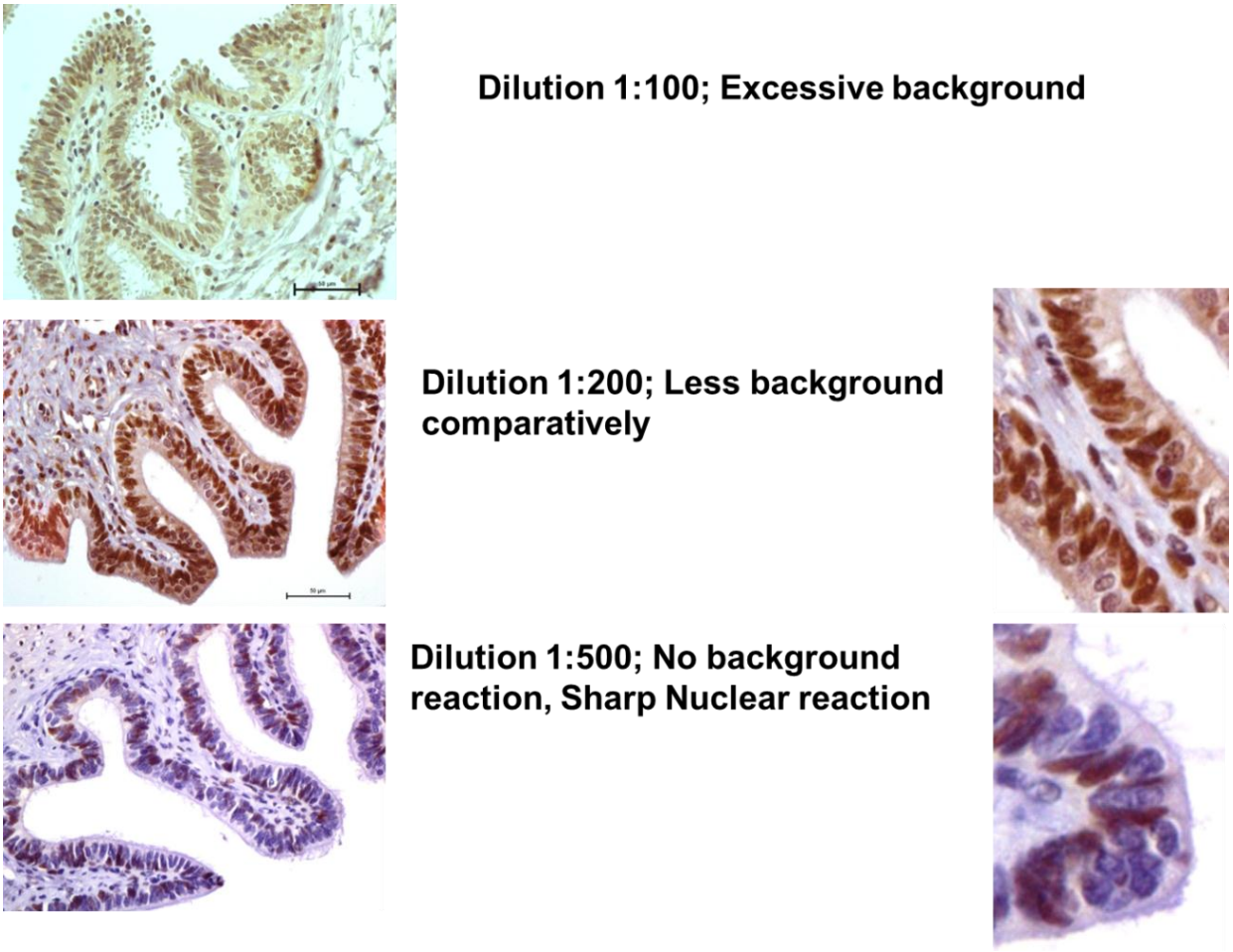


Fig. B. Photomicrographs showing immunoreactivity at different antibody dilutions

Final standardized Protocol

The sections in duplicate were mounted on super frost positively charged slides (Fisher Scientific). After dewaxing and rehydration was done the heat induced antigen retrieval was done in citrate buffer (AR 3 solution, Biogenex) and heating in microwave at 95^{o}C for 10 minutes and 98^{o}C for 5 minutes. Slides were then left for 30 min in hot buffer and washed in 0.1M phosphate buffered saline (at pH 7.4). The endogenous peroxidase activity was blocked by immersing the sections in 3% (v/v) H_2O_2 in methanol for 20 min followed by washing in 0.1M phosphate buffered saline (at pH 7.4). To prevent nonspecific binding of antibodies sections were blocked with normal horse serum (Vector's Laboratories USA). The sections were incubated with primary antibodies (ER and PR) at 4^{0}C for overnight staining box. After washing

in 0.1M phosphate buffered saline (at pH 7.4), the sections were incubated with universal secondary antibody (Vector Laboratories, USA). The chromogen used was 3, 3'-diaminobenzidine tetra hydrochloride (DAB) (Vector Laboratories, USA) with Gill's III haematoxylin counterstaining. The sections were washed in running tap water, dehydrated, cleared and mounted with DPX.

Image analysis

Immunostained sections were examined and photographed (10 images per slide per animal) using a light microscope (Nikon 80i) attached with a digital camera. The sections were evaluated semi-quantitavely by proportional scoring system by calculating the percentage of positively stained cell nuclei at 400 magnifications. These images were analysed using image analysis software (Image J).

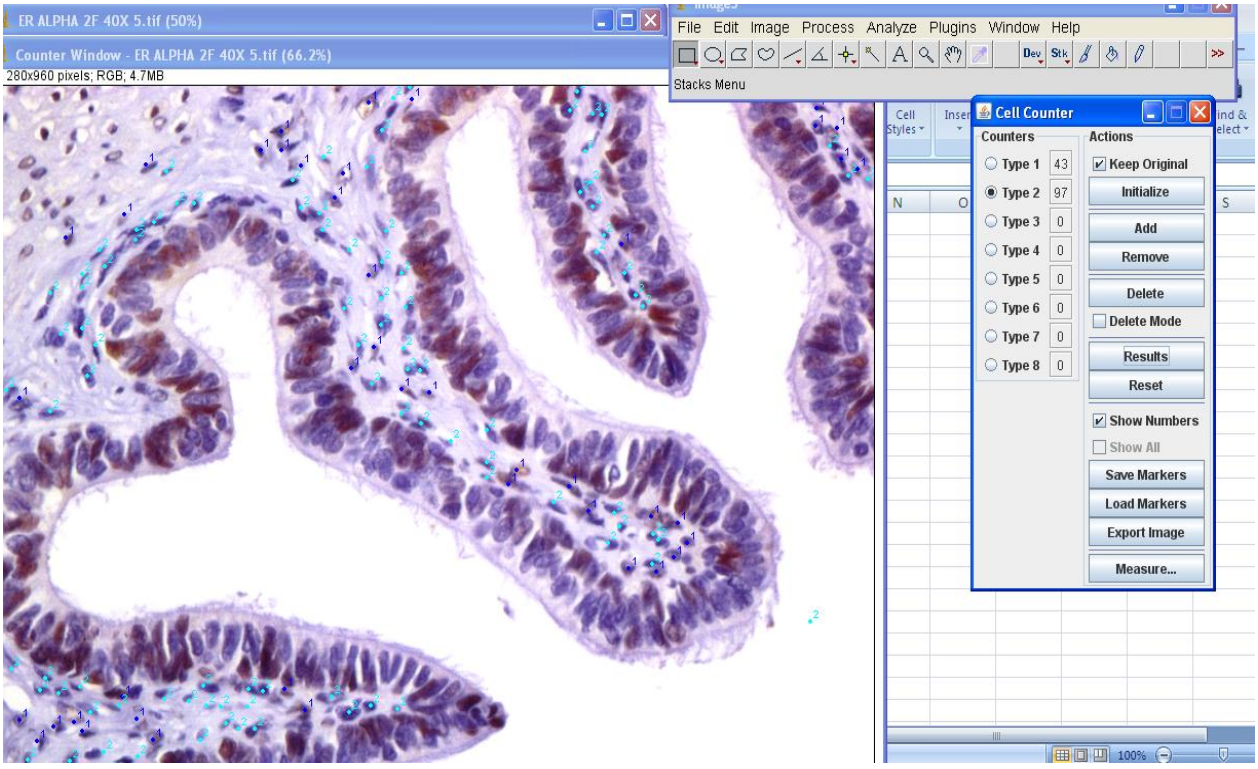


Fig.C. Counting of immunopositive and negative cells by ImageJ software

Experiment 2. Immunolocalization of Estrogen Receptor alpha (ERα) and progesterone receptor in Ovary buffalo

A. Estrogen Receptor alpha (ERα) in Ovary

ERα was localized in various cell types of buffalo ovary differentially in different stage of the reproductive cycle. Specific immunostaining was observed with anti-ERα antibody in the nuclei of follicular cells of primordial follicles (Fig. 1A), primary follicles (Fig.2B), Secondary (Fig.1C) and tertiary follicles (Fig.1D). No reaction was observed in ovarian surface epithelium. ERα immunostained cells were observed at the prepubertal, follicular phase, luteal phase and pregnant ovary. In primordial follicles nuclear reaction was observed in one or two granulosa cells of the follicle. Similar to the primordial follicles the ERα was localized the granulosa cells. The connective tissue around these follicles both in the superficial and deep stroma showed the strong reaction for ERα antibody. In the growing follicle and secondary follicle the reaction was strong. While in the tertiary follicles weak reaction was observed in the granulosa cells and theca cells.ERα was weak or absent in the cells of corpora lutea.

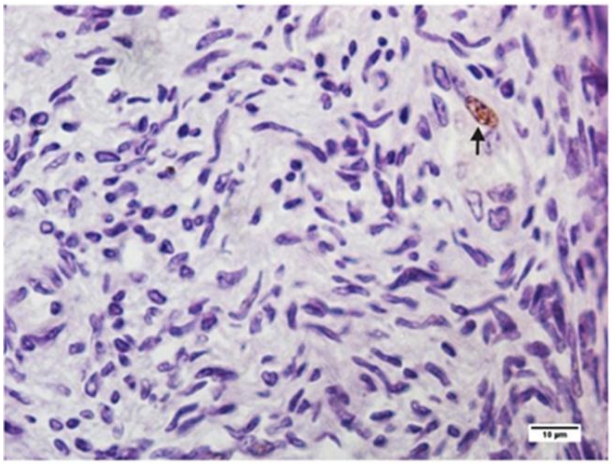


Fig.1A. Immunostaining of ovary of buffalo with anti-ERα antibody. Nuclear reaction in follicular cells of primordial follicle (arrow). Polymer HRP method. Original magnification x400

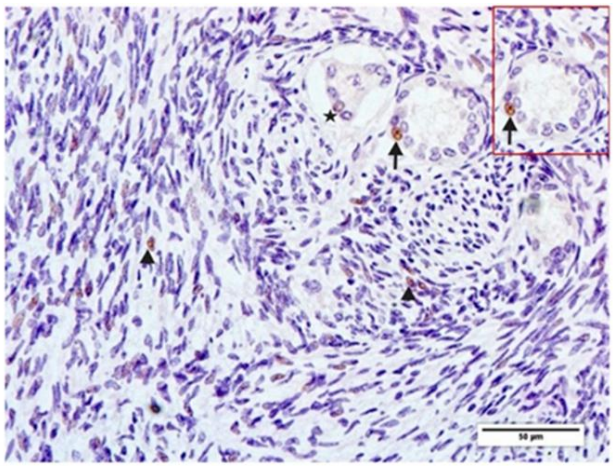


Fig.1B. Immunostaining of ovary of buffalo with anti-ERα antibody. Nuclear reaction in follicular cell of primary follicle (arrow), stromal cells (arrow head). Weak nuclear reaction in atretic follicle (star). Polymer HRP method. Original magnification x400

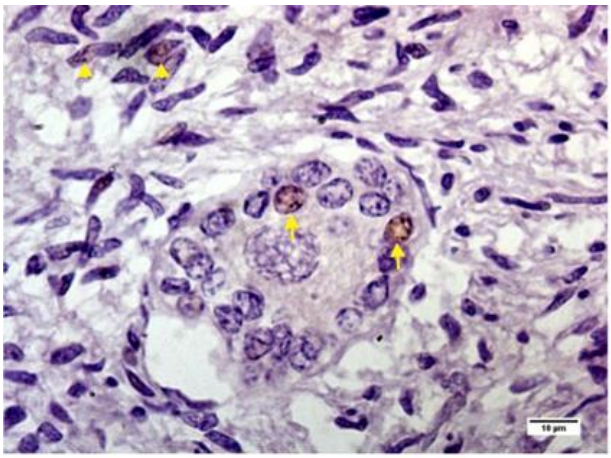


Fig.1C. Immunostaining of ovary of buffalo with anti-ERα antibody. Nuclear reaction in follicular cells of secondary follicle (arrow), stromal cells (arrow head). Polymer HRP method. Original magnification x400

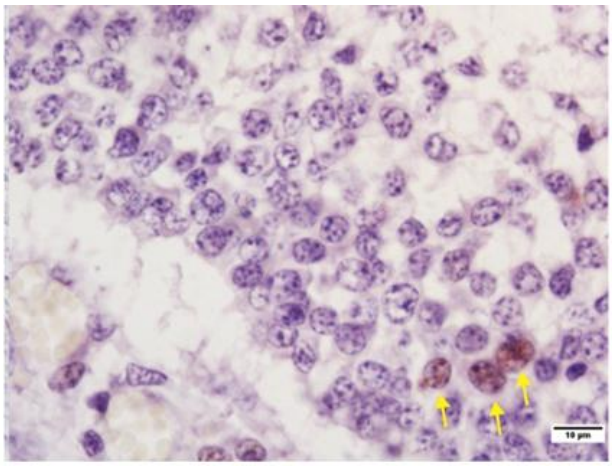


Fig.1D. Immunostaining of ovary of buffalo with anti-ERα antibody. Nuclear reaction in granulosa cells of tertiary follicle (arrow). Polymer HRP method. Original magnification x400

B. Progesterone Receptor (PRA) in Ovary

The progesterone receptors as revealed by immunohistochemistry were localized in the nuclei of different groups of ovarian cells. PR was localized in follicular cells of pre antral and antral follicles, stroma of ovary, endothelial cells of blood vessels. One or two granulosa cells of primodial and primary follicles were PR positive and immuoreaction was moderate while no staining was observed in oocytes (Fig.1E). Moderate nuclear reaction was observed in the stromal cells of ovarian cortex (Fig.1F&1G). In the antral follicles both granulosa cells as well as theca cells were immunostained for PR (Fig.1H). In the obliterative atretic follicles the invading stromal cells were highly positive for PR (Fig.1I.). No staining was observed in negative controls (Fig. 1J).

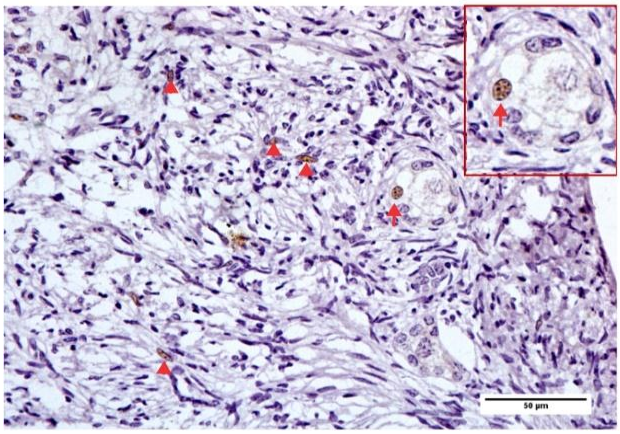


Fig.1E. Immunostaining of ovary of buffalo with anti-PRA antibody. Nuclear reaction in follicular cells of primordial follicle (arrow), stromal cells (arrow head). Polymer HRP method. Original magnification x400 Inset showing magnified view of nuclear reaction in follicular cell (arrow)

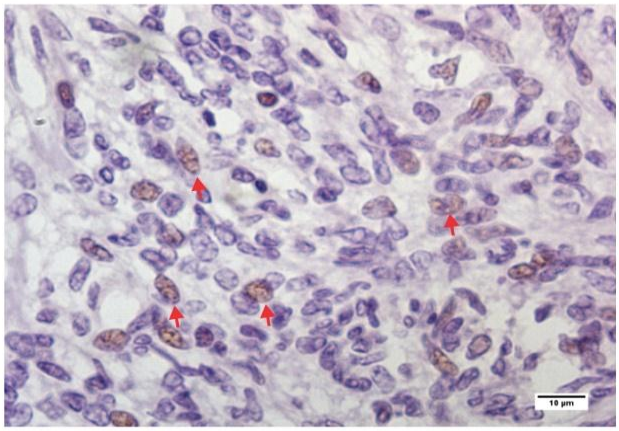


Fig.1F. Immunostaining of ovary of buffalo with anti-PRA antibody. Nuclear reaction in follicular cells of primordial follicle (arrow). Polymer HRP method. Original magnification x400

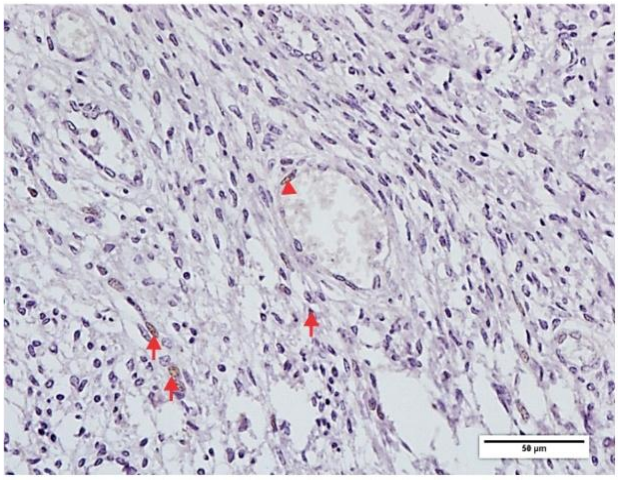


Fig.1G. Immunostaining of ovary of buffalo with anti-PRA antibody. Nuclear reaction in stromal cells (arrow) and endothelial cells (arrow head). Polymer HRP method. Original magnification x400

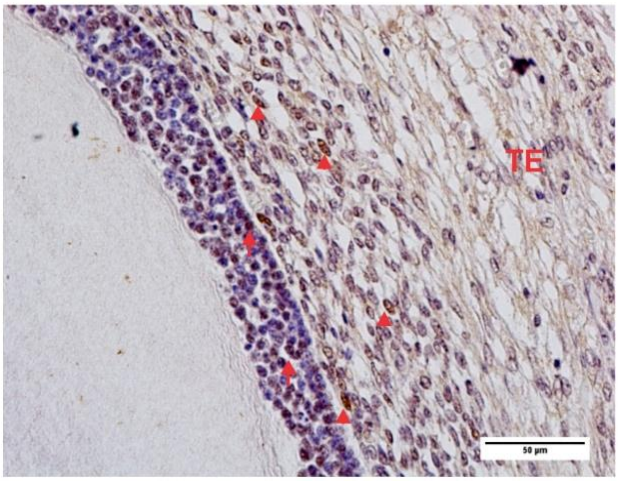


Fig.1H. Immunostaining of ovary of buffalo with anti-PRA antibody. Nuclear reaction in granulosa cells of tertiary follicles (arrow), theca interna cells (arrow) and theca externa cells (TE). Polymer HRP method. Original

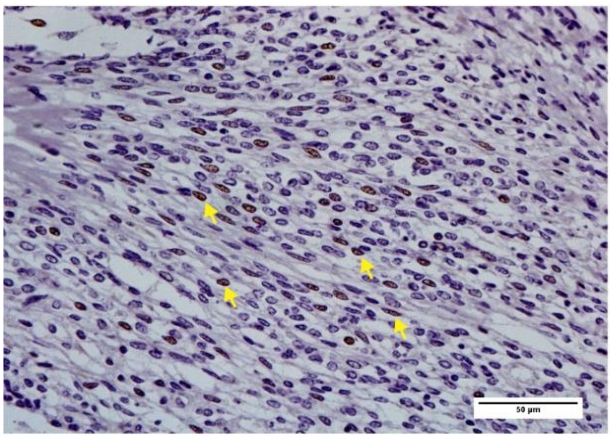


Fig.1I. Immunostaining of ovary of buffalo with anti-PRA antibody. Nuclear reaction in stromal cells (arrow) in obliterative atretic follicle. Polymer HRP method. Original magnification x400

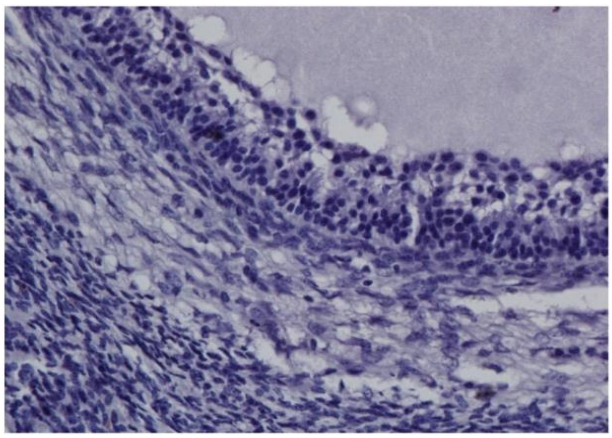
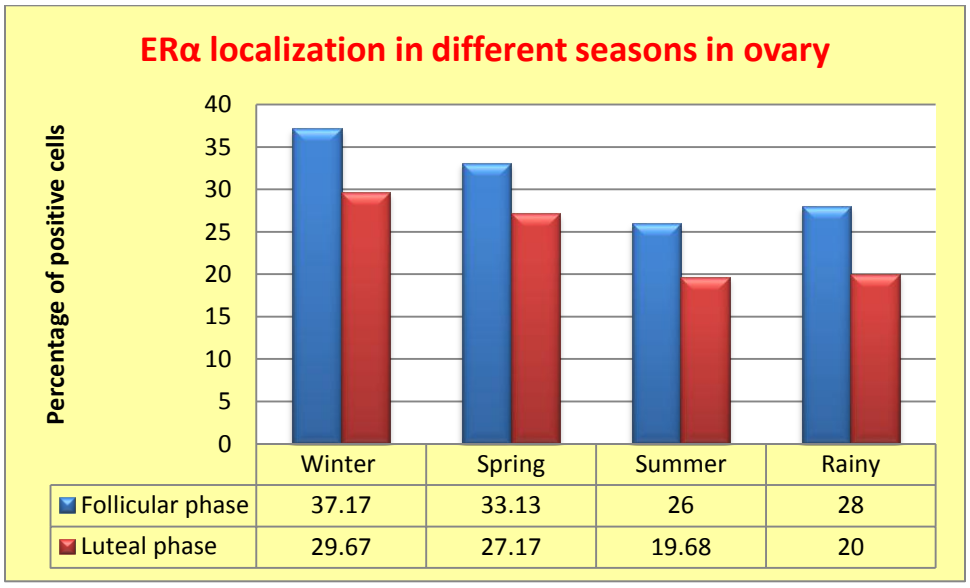


Fig.1J. Negative control for immunostaining anti-PRA antibody. Polymer HRP method. Original magnification x400

Seasonal Variation in ERα and PR expression:

Both the receptors showed seasonal variation in their expression pattern. Localization of both the receptors during the winter and spring seasons were higher as compared to that of summer and rainy season.



	Winter	Spring	Summer	Rainy
Follicular phase	37.17	33.13	26	28
Luteal phase	29.67	27.17	19.68	20

Experiment 3. Immuno localization of estrogen receptor in oviduct of buffalo during follicular and luteal phases of estrous cycle

A. Immuno localization of estrogen receptor in oviduct of buffalo

With Immunohistochemical staining oviducts from twelve buffaloes were screened for the presence of estrogen receptor alpha (ERα) during follicular and luteal phase of estrous cycle. ERs were detected on paraffin sections using one step poly HRPO staining technique and the reactivity was scored semi-quantitatively. The positive immune staining was detected in the nuclei of the cells. ERα was observed in infundibulum, ampulla, and isthmus of buffalo oviduct. The nuclei of epithelial, stromal, and smooth muscle cells showed nuclear localization of estrogen receptors (Fig.1). Comparable staining intensities and distributions of estrogen receptor localization were detected in different portions of the oviduct. The serosa, endothelial cells of blood vessels and the connective tissues in serosa had only few cells staining positive for estrogen receptor. Overall, the intensity of staining for ER was strong in oviduct during follicular phase. The results indicated that oviductual ERα in buffalo varied according to the phase of the ovarian activity.

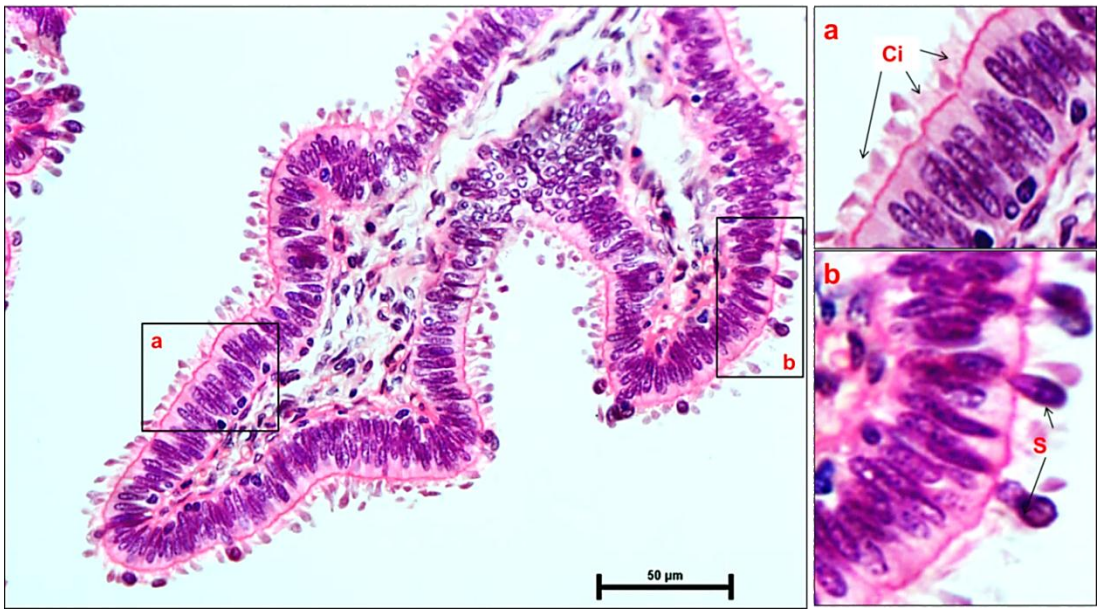


Fig.1. Paraffin section of oviduct (Infundiblum) of buffalo showing mucosal folds lined with columnar cells. Columnar cells were either ciliated cell (Inset a) and secretory cells (inset b). Haematoxylin and eosin stain x400.

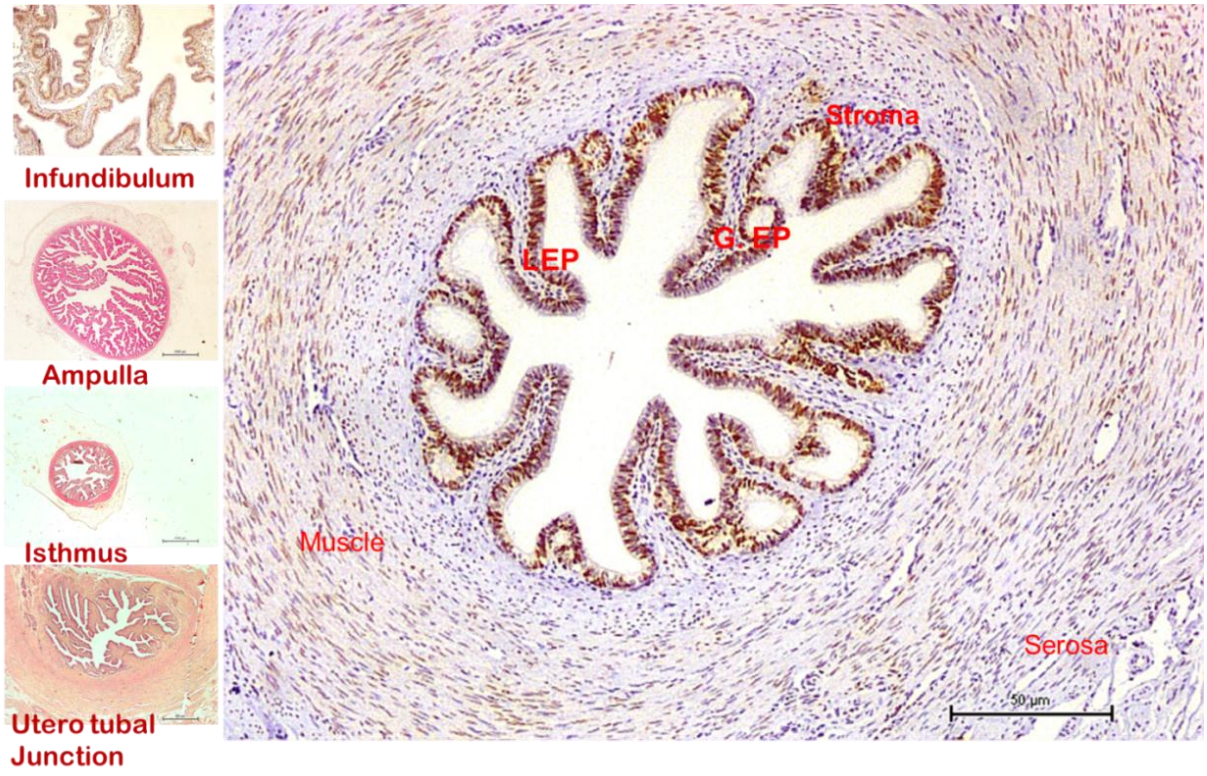


Fig.2. Immunolocalization of ER in different regions of oviduct and different compartments of oviduct (Tunica mucosa, T. muscularis and T. Serosa)

- Immunoreactive ER α was localized to nuclei of the luminal epithelial cell layer, stromal cells and muscle layer cells in cross sections of buffalo oviducts during both the phases of estrous cycle.
- Faint cytoplasmic staining was observed in all the segments of oviduct more during follicular phase than the luteal phase of estrous cycle.
- Study demonstrated that ER α localization during follicular phase than during luteal phase in buffalo oviduct suggesting that
- Upregulation of ERα was observed during the follicular phase and could be correlated with enhanced ciliary and secretory activity of the epithelium.
- Stromal estrogen receptor localization trigger the responsiveness of the epithelium to the steroid hormones.

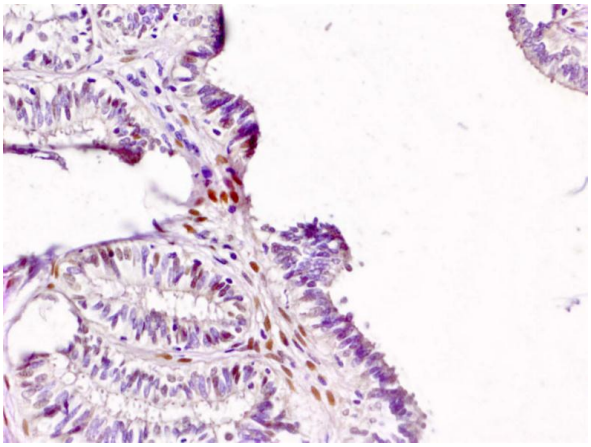


Fig.3A. Immunostaining of infundibulum of buffalo with anti-ERα antibody. Nuclear reaction in lining epithelium and lamina propria cells of oviduct. Polymer HRP method. Original magnification x100

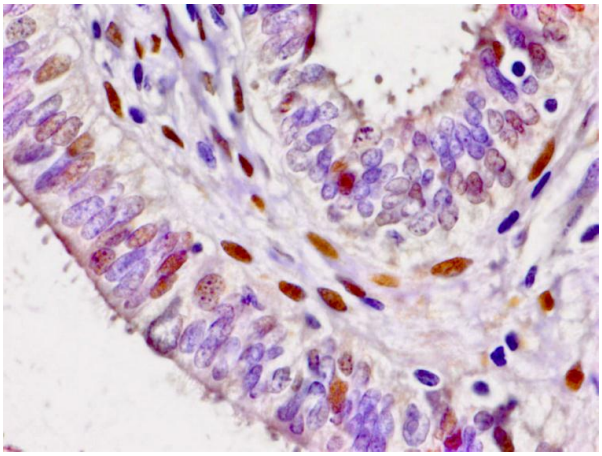


Fig.3B. Immunostaining of infundibulum of buffalo with anti-ERα antibody. Nuclear reaction in ciliated cells and lamina propria cells of oviduct. Polymer HRP method. Original magnification x400

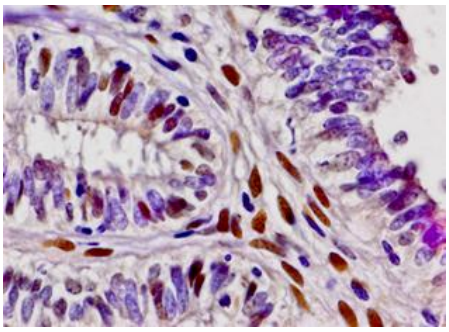


Fig.3C. Immunostaining of infundibulum of buffalo with anti-ERα antibody. Nuclear reaction in oviductal glands and lamina propria cells of oviduct. Polymer HRP method. Original magnification x400

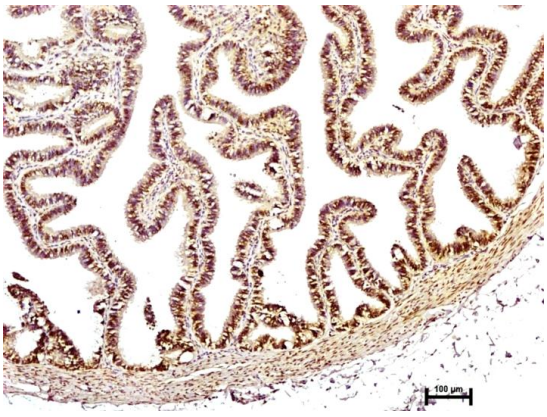


Fig.3D. Immunostaining of ampulla of buffalo with anti-ERα antibody. Nuclear reaction in lining epithelium and propria submucosa cells, tunica muscularis and T. serosa of oviduct. Polymer HRP method. Original magnification x100

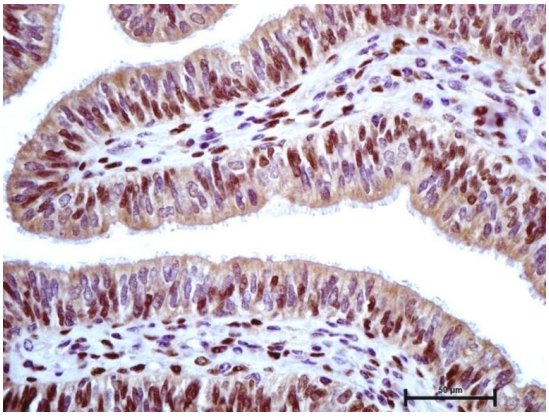


Fig.3E. Immunostaining of ampulla of buffalo with anti-ERα antibody. Nuclear reaction in lining epithelium and propria submucosa cells. Polymer HRP method. Original magnification x400

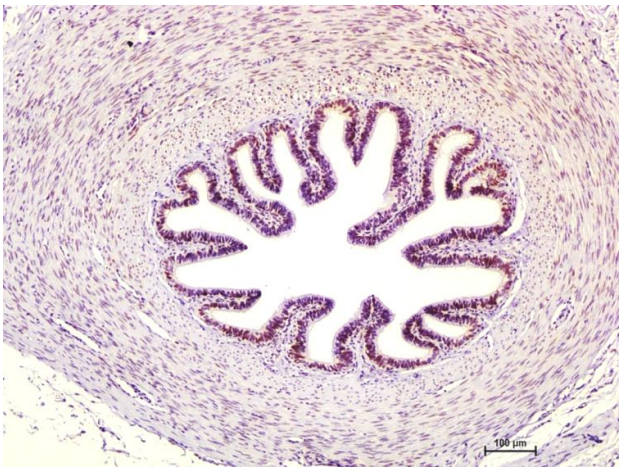


Fig.3F. Immunostaining of Isthmus of buffalo with anti-ERα antibody. Nuclear reaction in lining epithelium and propria submucosa cells, tunica muscularis and T. serosa of oviduct. Polymer HRP method. Original magnification x100

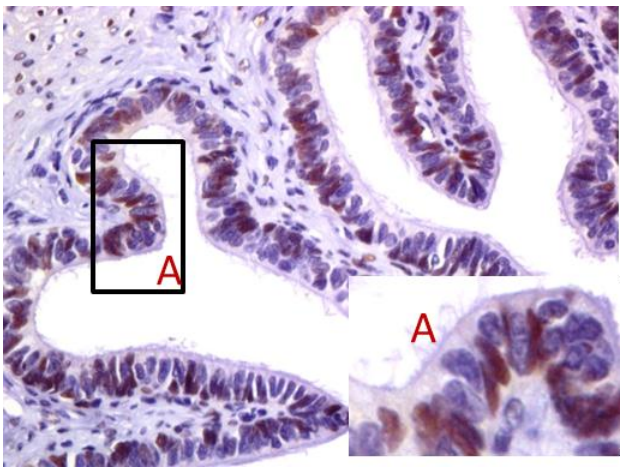


Fig.3G. Immunostaining of isthmus of buffalo with anti-ERα antibody. Nuclear reaction in lining epithelium and propria submucosa cells. Polymer HRP method. Original magnification x400

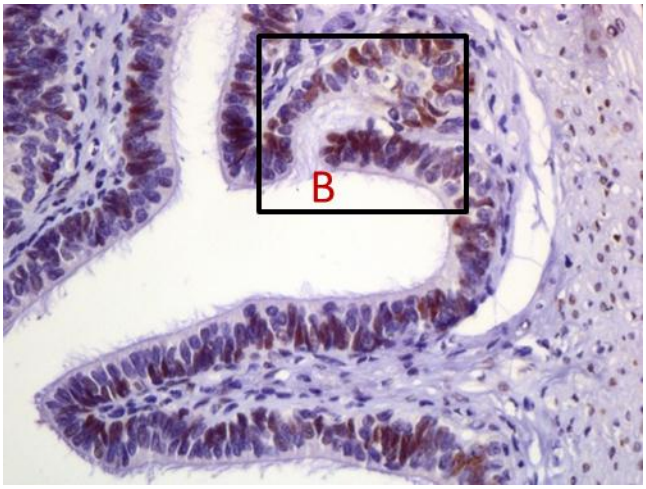


Fig.3H. Immunostaining of isthmus of buffalo with anti-ERα antibody. Nuclear reaction in lining epithelium and propria submucosa cells. Polymer HRP method. Original magnification x400

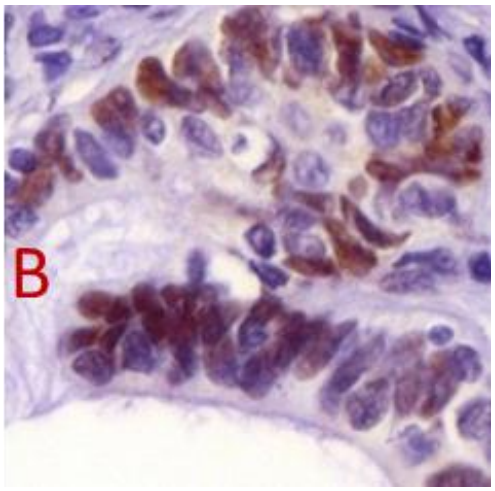


Fig.3I. Magnified view of B portion of Fig.3H showing immunostaining oviductal gland with anti-ERα antibody.

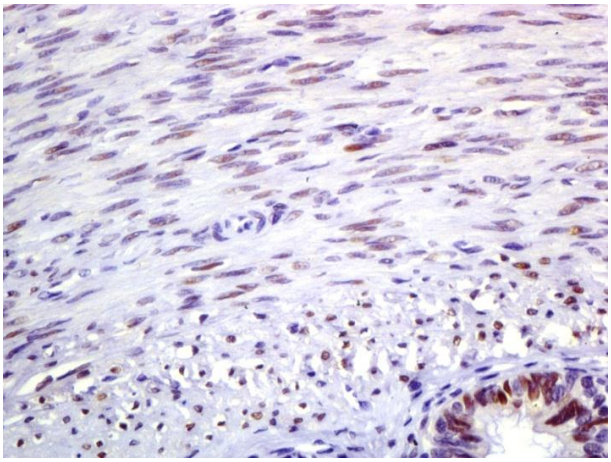


Fig.3J. Immunostaining of isthmus of buffalo with anti-ERα antibody. Nuclear reaction in tunica muscularis Polymer HRP method. Original magnification x400

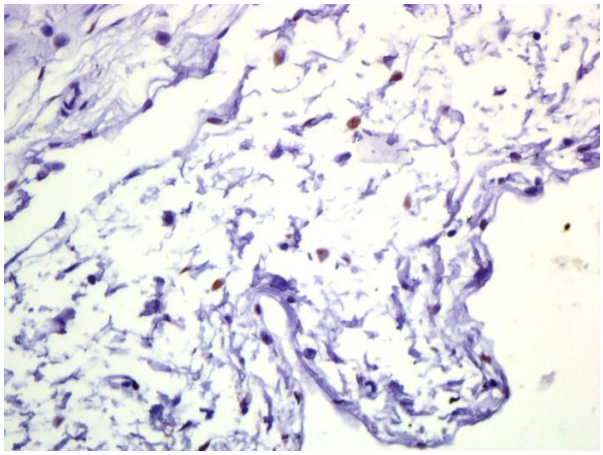


Fig.3K. Immunostaining of isthmus of buffalo with anti-ERα antibody. Nuclear reaction in tunica serosa. Polymer HRP method. Original magnification x400

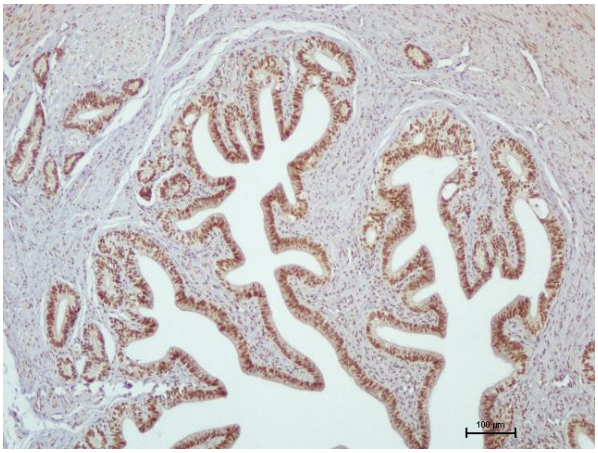


Fig.3L. Immunostaining of isthmus of buffalo with anti-ERα antibody. Nuclear reaction in lining epithelium and propria submucosa cells. Polymer HRP method. Original magnification x400

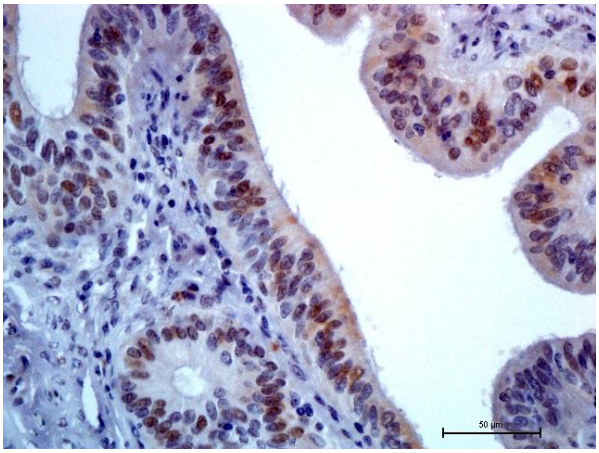


Fig.3L. Immunostaining of isthmus of buffalo with anti-ERα antibody. Nuclear reaction in lining epithelium and propria submucosa cells. Polymer HRP method. Original magnification x400

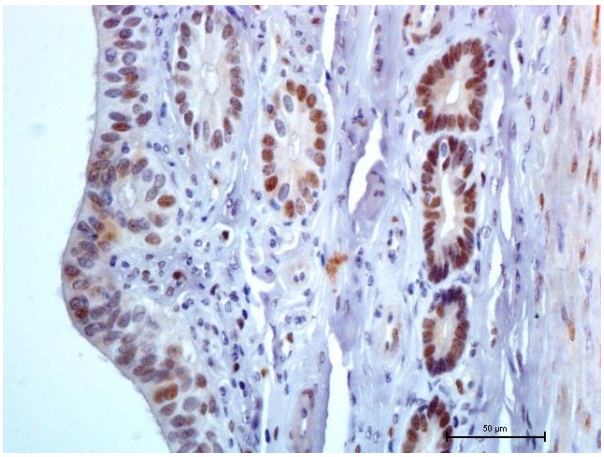


Fig.3H. Immunostaining of isthmus of buffalo with anti-ERα antibody. Nuclear reaction in tunica muscularis Polymer HRP method. Original magnification x400

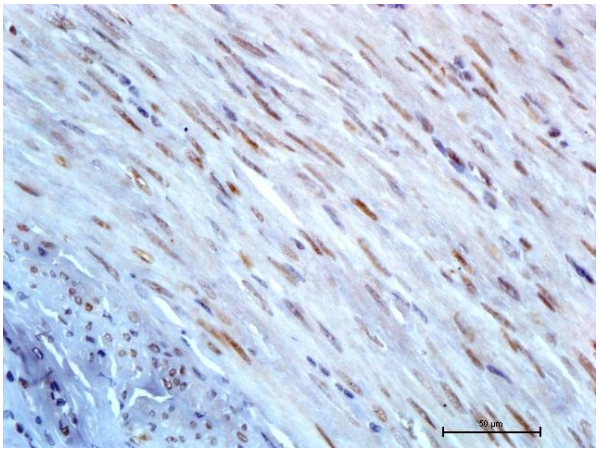


Fig.3H. Immunostaining of isthmus of buffalo with anti-ERα antibody. Nuclear reaction in tunica muscularis Polymer HRP method. Original magnification x400

Figure 3I shows the quantitative analysis of immunopositive cells. The data showed that highest immune positive cells were observed in ampulla region of oviduct and was lowest in the utero-tubal junction ($p<0.05$). Ampulla showed statistically significant higher number of ERα positive cells as compared to the all the segments of oviduct. Infundibulum, ampulla and isthmus showed higher percentage of ERα positive cells during follicular phase of estrous cycle as compared to the luteal phase of estrous cycle ($p<0.05$). There was no significant difference in the percentage positive cells during two phases of estrous cycle in the utero-tubal junction.

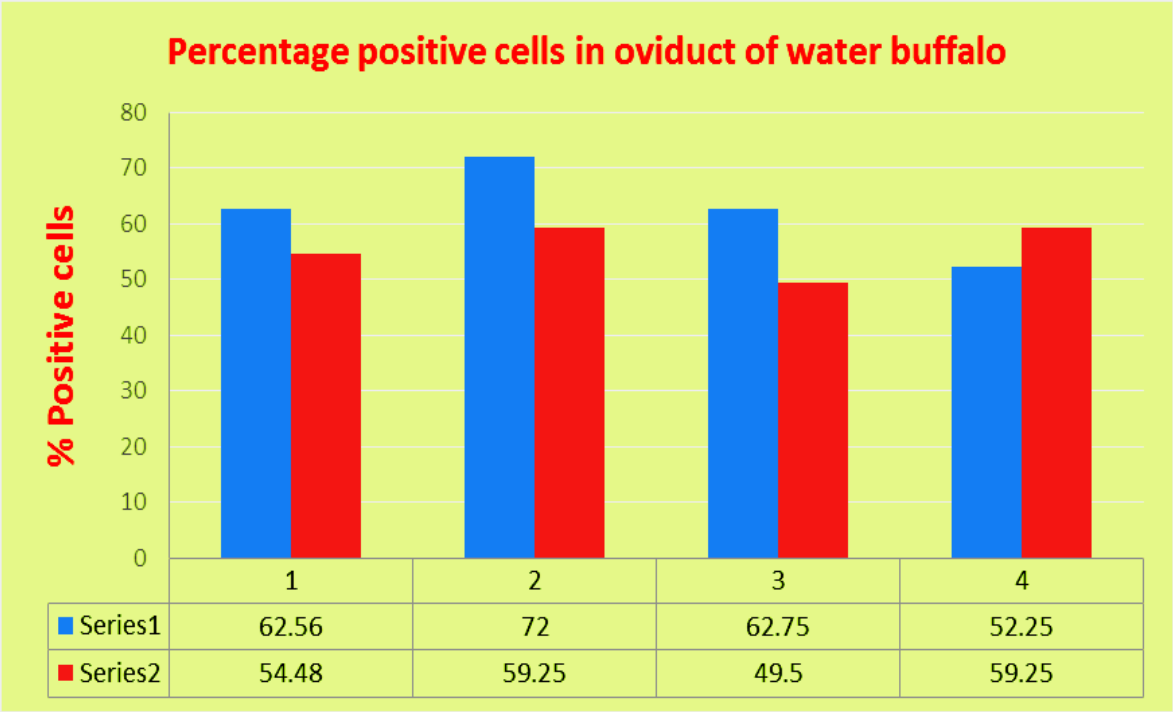


Fig. 3I. Graph showing percentage of ERα positive cells in different segments of oviduct during follicular and luteal phase of estrous cycle.

B. Immuno localization of progesterone receptor in oviduct of buffalo

The steroid hormone, progesterone, is a key modulator of normal reproductive functions of animals. The present investigation aimed at determination of distribution of progesterone receptor in different segments of oviduct of buffalo during follicular and luteal phases of estrous cycle. Oviducts from twelve buffaloes (six each during follicular and luteal phases) were collected from slaughter house. Blood samples were also collected from before slaughter of the animal to estimate levels of estrogen and progesterone hormones. The tissue distribution of progesterone receptor was examined using immuno histochemical technique (one step super sensitive polymer based horse radish peroxidases).

- The progesterone receptor was localized in lamina epithelialis, propria submucosa, tunica muscularis and tunica serosa.
- The maximum localization was observed in lamina epithelialis where both ciliated and secretory cell types were positive for progesterone receptors. Percentage of positive cells varied during the follicular and luteal phases of estrous cycle.

- The lining epithelium of oviductal glands formed due to in folding of mucosal folds or joining of the two mucosal adjacent folds were also intensely positive for progesterone receptor.

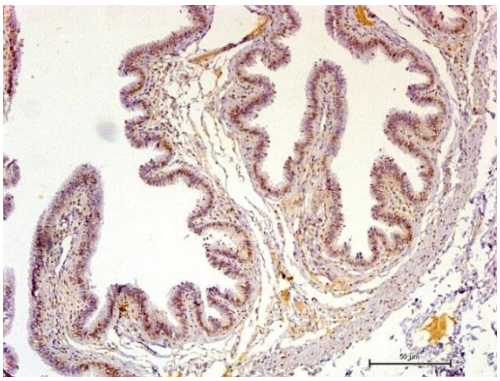


Fig.3A. Immunostaining of infundibulum of buffalo with anti-PR antibody. Nuclear reaction in tunica mucosa, propria submucosa, tunica muscularis and tunica serosa. Polymer HRP method. Original magnification x400

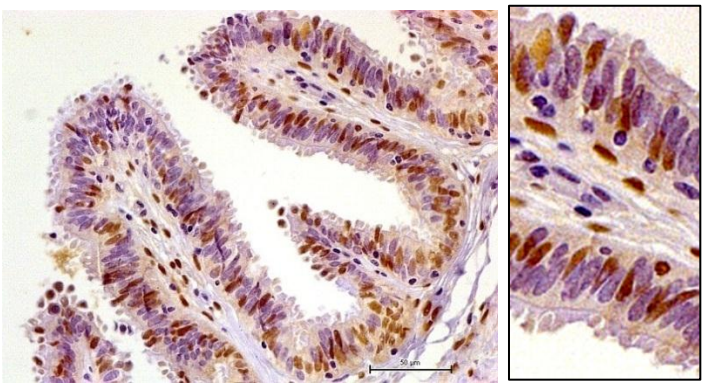


Fig.3B. Immunostaining of infundibulum of buffalo with anti-PR antibody. Nuclear reaction in tunica muscularis. Polymer HRP method. Original magnification x400

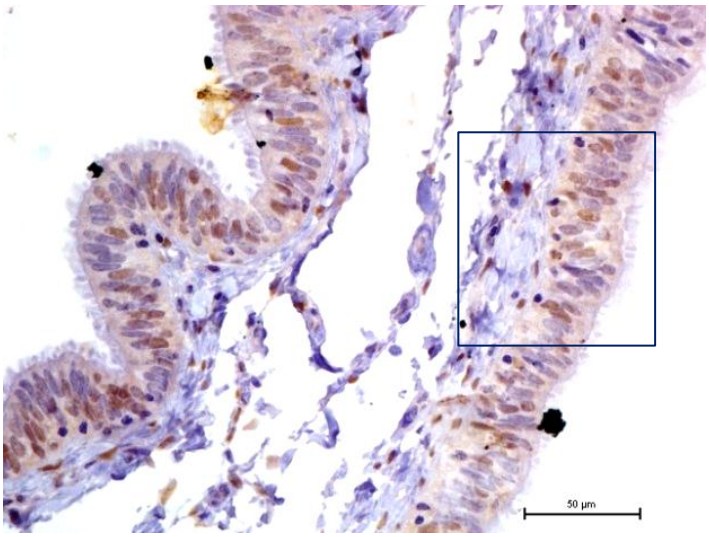


Fig.3C. Immunostaining of infundibulum of buffalo with anti-PR antibody. Nuclear reaction in tunica mucosa and Propria submucosa. Polymer HRP method. Original magnification x400

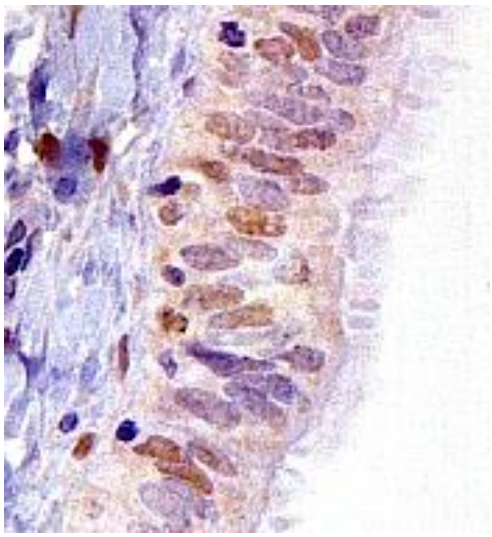


Fig.3D. Magnified view of immunostaining of lamina epithelialis of image 3C. Nuclear reaction in ciliated and secretory cells. Polymer HRP method. Original magnification x400

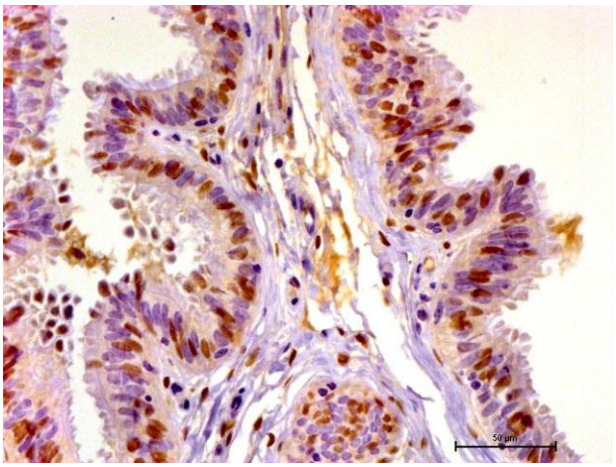


Fig.3E. Immunostaining of infundibulum of buffalo during luteal phase with anti-PR antibody. Nuclear reaction in tunica mucosa and Propria submucosa. Polymer HRP method. Original magnification x400

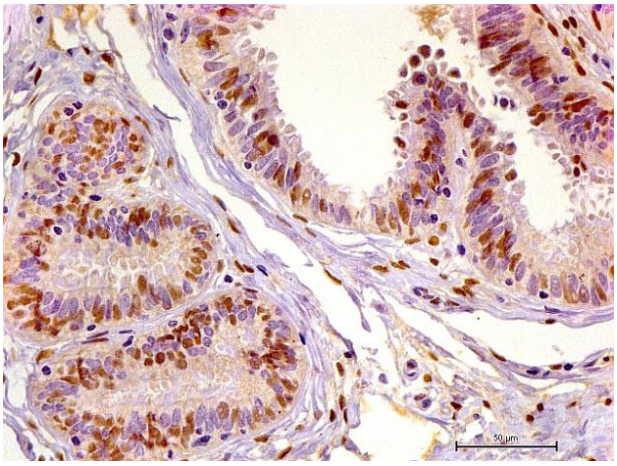


Fig.3F. Immunostaining of infundibulum of buffalo during luteal phase with anti-PR antibody. Nuclear reaction in tunica mucosa and Propria submucosa and oviductual glands. Polymer HRP method. Original magnification x400

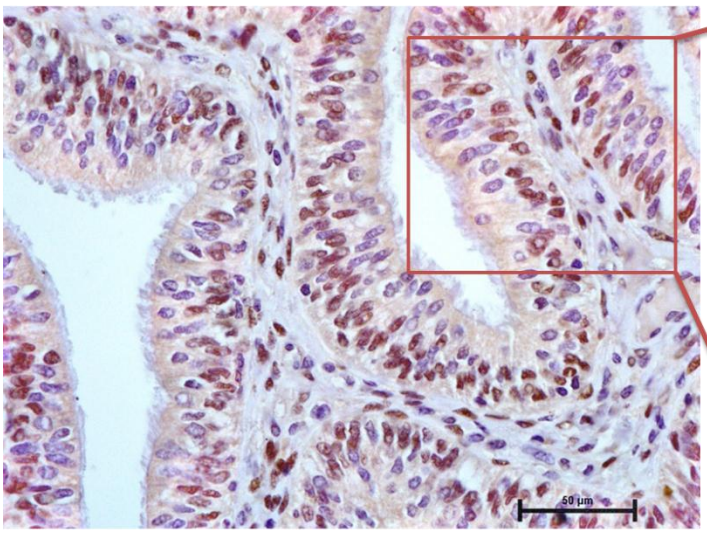


Fig.3G. Immunostaining of ampulla of buffalo with anti-PR antibody. Nuclear reaction in tunica mucosa and Propria submucosa. Polymer HRP method. Original magnification x400

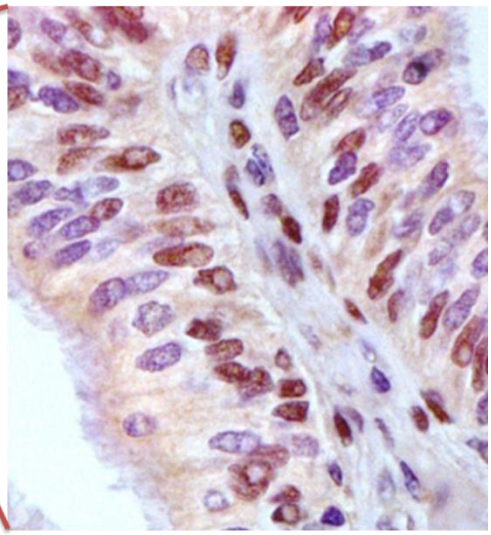


Fig.3H. Magnified view of selected area of image 3G showing nuclear reaction in tunica mucosa and Propria submucosa.

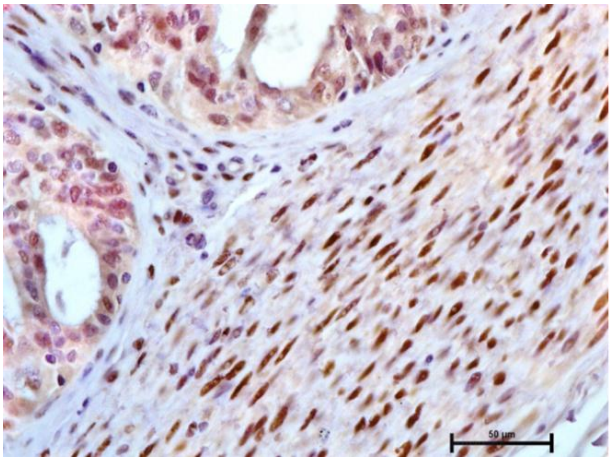


Fig.3I. Immunostaining of ampulla of buffalo with anti-PR antibody. Nuclear reaction in propria submucosa and tunica muscularis. Polymer HRP method. Original magnification x400

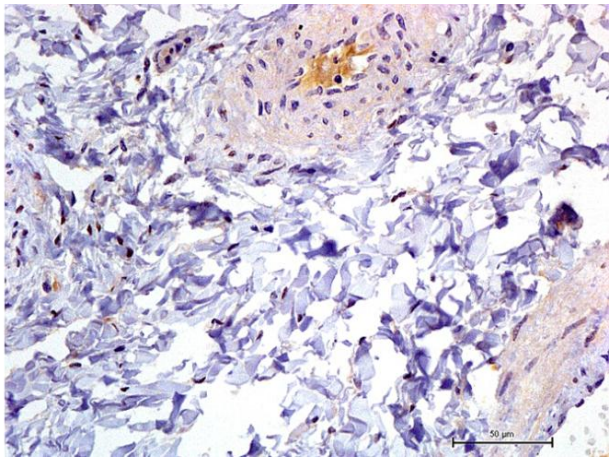


Fig.3J. Immunostaining of infundibulum of buffalo with anti-PR antibody. Nuclear reaction in tunica serosa and blood vessel. Polymer HRP method. Original magnification x400

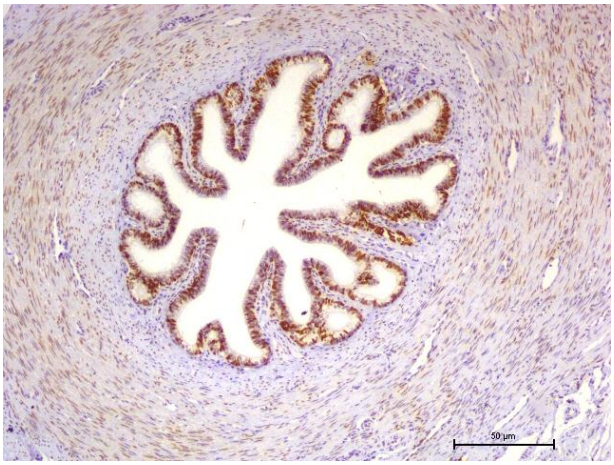


Fig.3K. Immunostaining of isthmus of buffalo with anti-PR antibody. Nuclear reaction in tunica muscularis Polymer HRP method. Original magnification x400

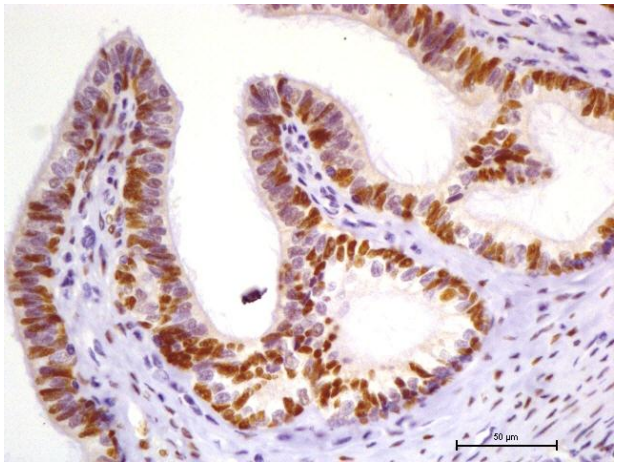


Fig.3K. Immunostaining of isthmus of buffalo with anti-PR antibody. Nuclear reaction in tunica muscularis Polymer HRP method. Original magnification x400

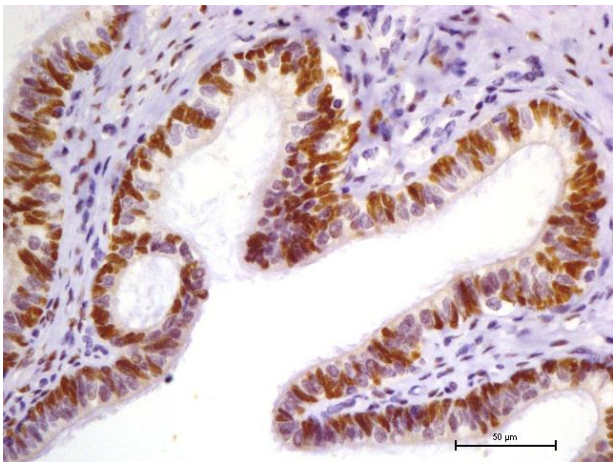


Fig.3K. Immunostaining of isthmus of buffalo with anti-PR antibody. Nuclear reaction in tunica muscularis Polymer HRP method. Original magnification x400

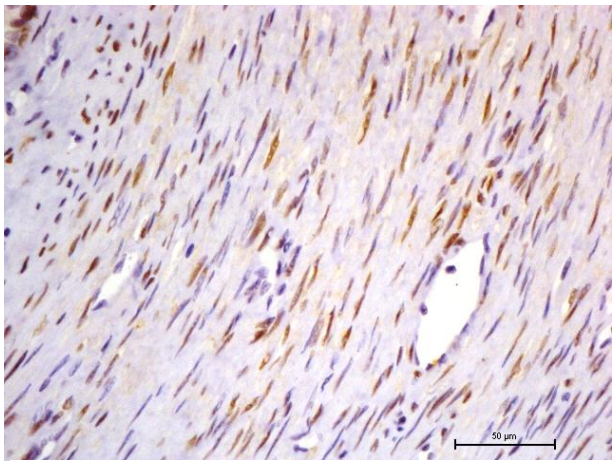


Fig.3K. Immunostaining of isthmus of buffalo with anti-PR antibody. Nuclear reaction in tunica muscularis Polymer HRP method. Original magnification x400

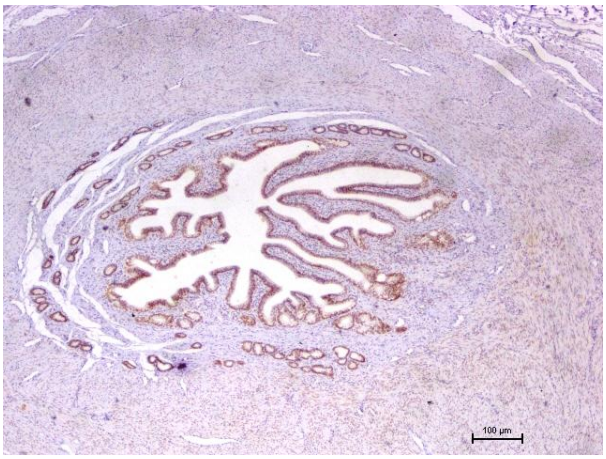


Fig.3M. Immunostaining of uterotubal junction of buffalo during follicular phase with anti-PR antibody. Nuclear reaction in tunica mucosa, Propria submucosa, tunica muscularis and tunica serosa. Polymer HRP method. Original magnification x40

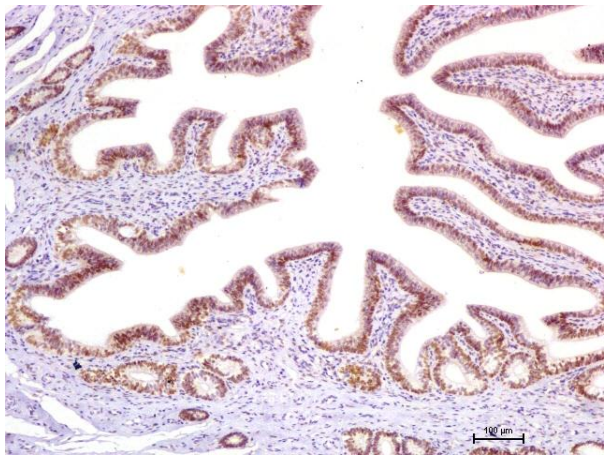


Fig.3M. Immunostaining of uterotubal junction of buffalo during follicular phase with anti-PR antibody. Nuclear reaction in tunica mucosa and Propria submucosa oviductual glands. Polymer HRP method. Original magnification x100

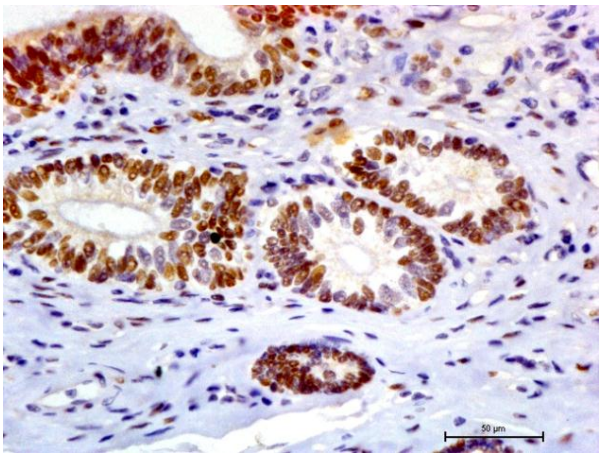


Fig.3M. Immunostaining of uterotubal junction of buffalo during follicular phase with anti-PR antibody. Nuclear reaction in tunica mucosa, Propria submucosa, and glandular epithelium. Polymer HRP method. Original magnification x400

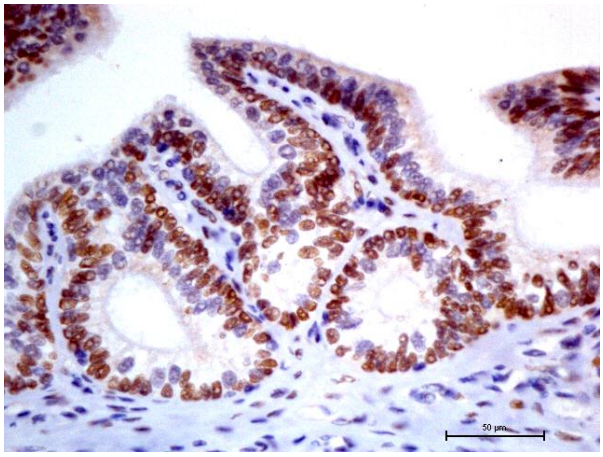


Fig.3M. Immunostaining of uterotubal junction of buffalo during follicular phase with anti-PR antibody. Nuclear reaction in tunica mucosa and Propria submucosa, and glandular epithelium. Polymer HRP method. Original magnification x400

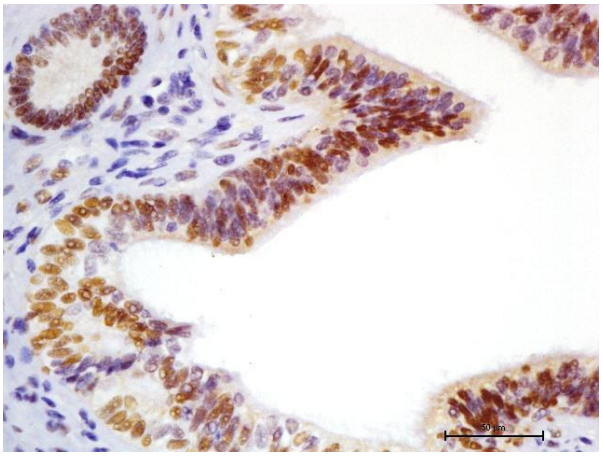


Fig.3M. Immunostaining of uterotubal junction of buffalo during luteal phase with anti-PR antibody. Nuclear reaction in tunica mucosa and Propria submucosa, and glandular epithelium. Polymer HRP method. Original magnification x400

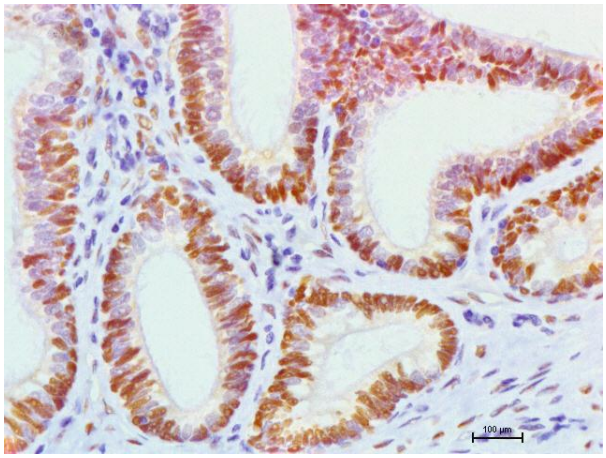


Fig.3M Immunostaining of uterotubal junction of buffalo during luteal phase with anti-PR antibody. Nuclear reaction in Propria submucosa, and glandular epithelium. Polymer HRP method. Original magnification x400

C. Electron microscopic and Immuno electron microscopic studies on oviduct of buffalo

Transmission electron microscopic study revealed that mucosa of oviduct was lined with ciliated and secretory cells as also observed in the paraffin sections stained with the haematoxylin and eosin staining. Ciliated cells had bunch of cilia emerged from their apical surface (Fig. 3N and 3O) while secretory cells showed secretory blebs on their apical surface. Immunogold labeling with anti ERα antibody demonstrated immunoglod particles both in the cytoplasm (3P) and nucleus (3Q).

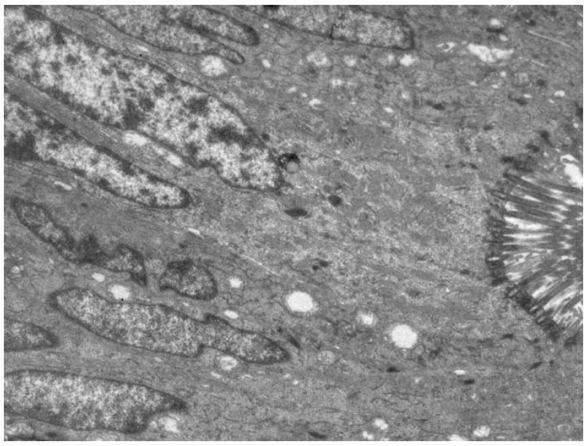


Fig.3N. TEM photograph showing apical portion of epithelium of infundibulum at follicular phase showing ciliated cell (cc) with many mature cilia (ci)

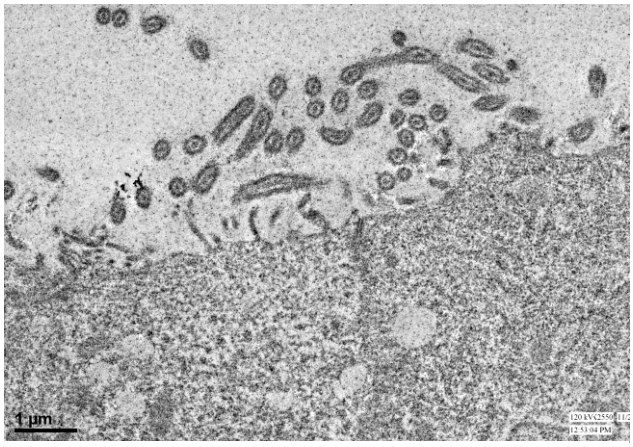


Fig.3O. TEM photograph showing apical portion of epithelium of infundibulum at follicular phase showing ciliated cell (cc) with many mature cilia (ci)

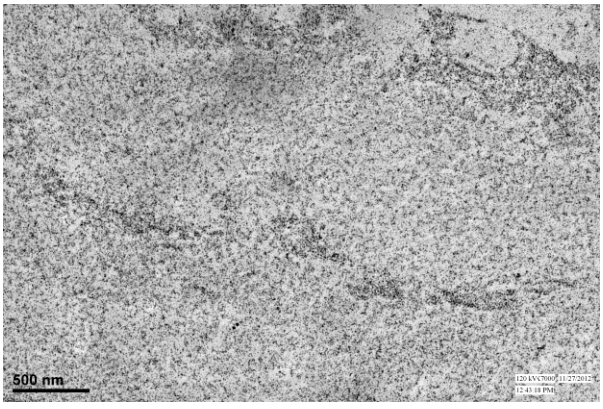


Fig.3P. Transmission electron micrograph showing immune labeling with ERα antibody in epithelium of infundibulum at follicular phase showing immunogold particles in cytoplasm.

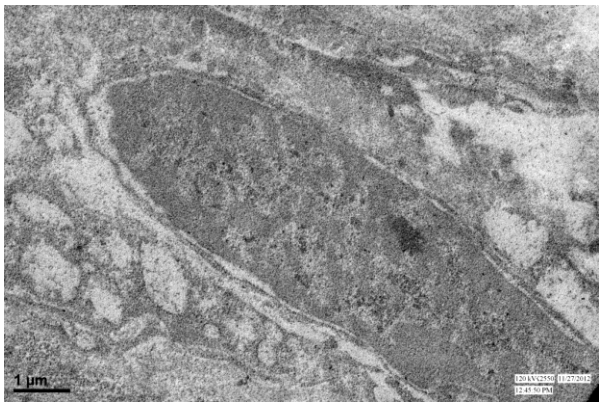


Fig.3Q. Transmission electron micrograph showing immune labeling with ERα antibody in epithelium of infundibulum at follicular phase showing immunogold particles in the nucleus.

Immunogold labeling was seen dispersed in cytoplasm in different organelle, mitochondria and rough endoplasmic reticulum (Fig.3R). It was also localized in the cilia (Fig.3S and 3T) and thus could be responsible for the beating of cilia.

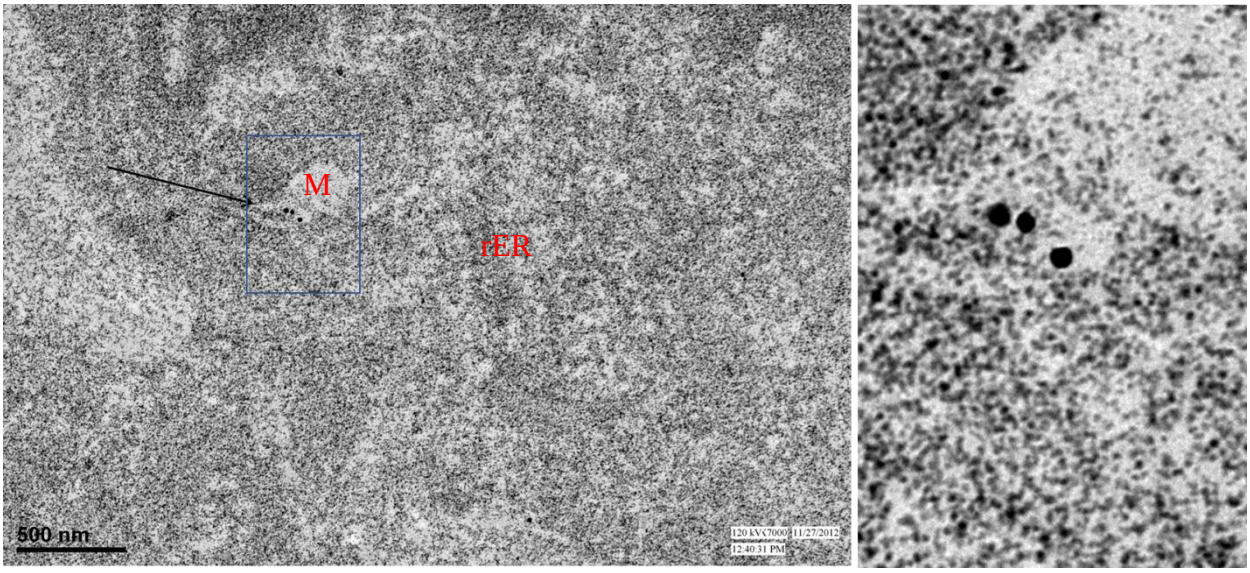


Fig.3R. Fig.3P. Transmission electron micrograph showing immune labeling with ERα antibody in epithelium of infundibulum at follicular phase showing immunogold particles in mitochondria (M) and rough endoplasmic reticulum (rER).

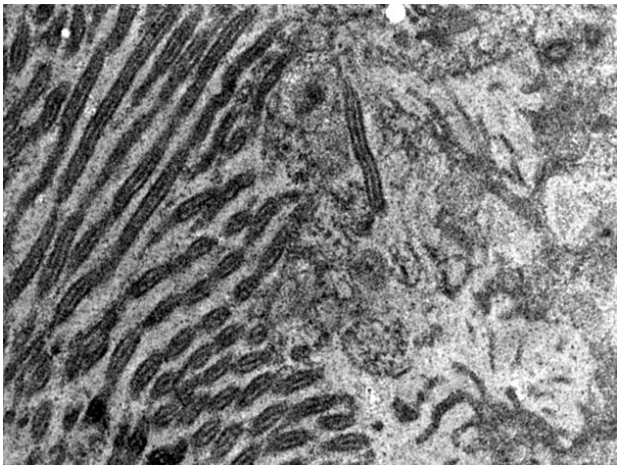


Fig.3S. Transmission electron micrograph showing immune labeling with ERα antibody in epithelium of infundibulum at follicular phase showing immune gold particles in longitudinal sections of cilia.

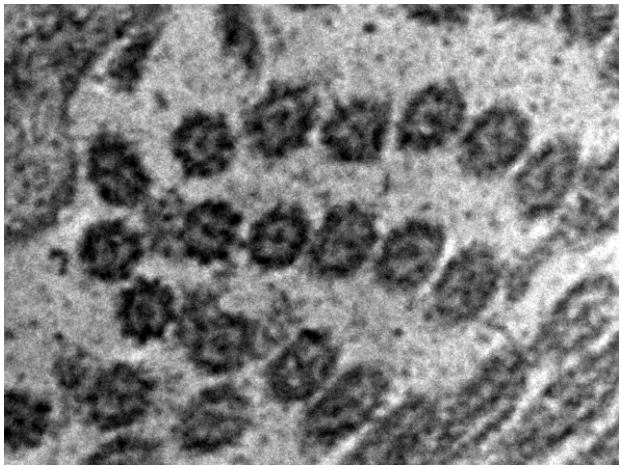
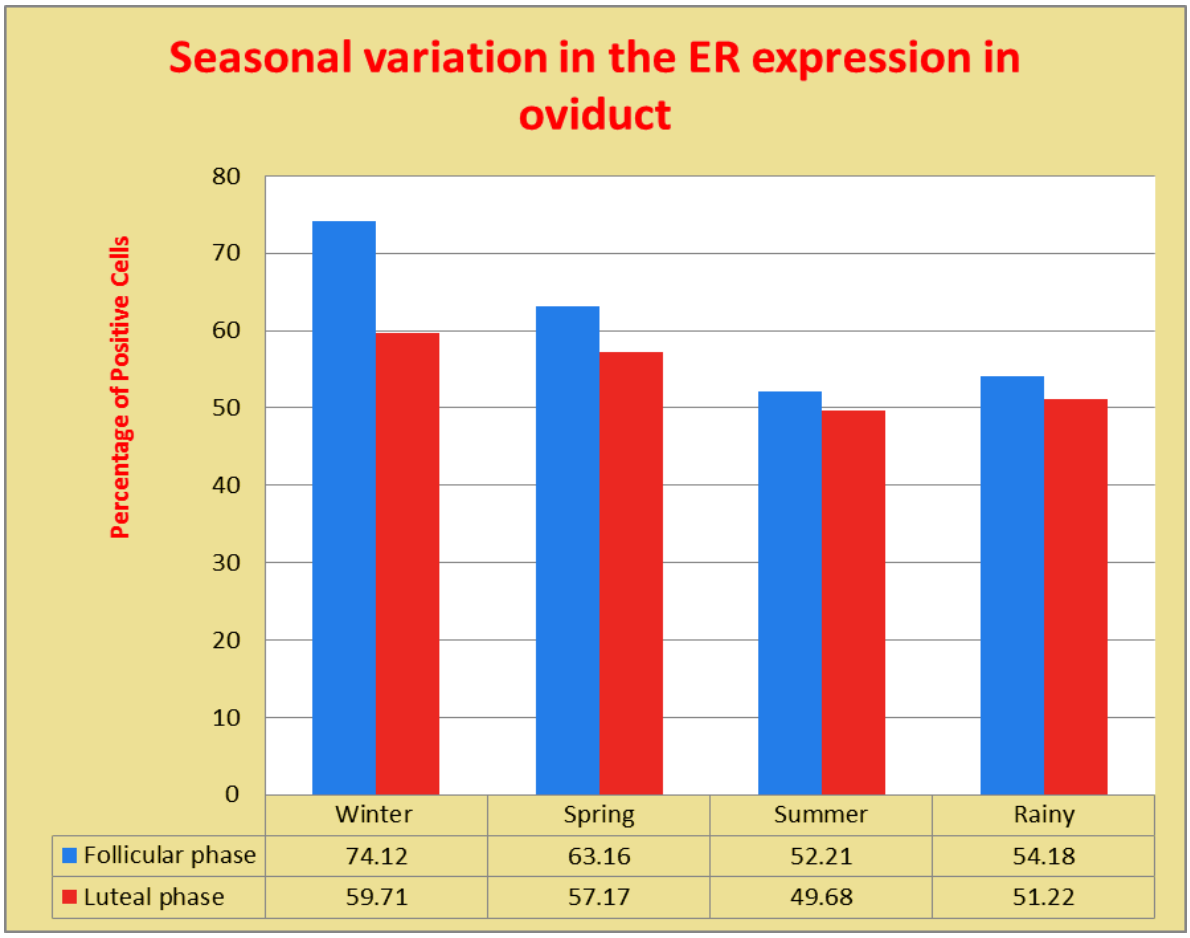


Fig.3T. Transmission electron micrograph showing immune labeling with ERα antibody in epithelium of infundibulum at follicular phase showing immune gold particles in cross sections of cilia.

Seasonal variation in the immunoexpression pattern of ERαin oviduct of buffalo:

The data and analysis of immuno expression of ERα in oviduct of buffalo during winter, spring, summer and rainy seasons has been presented in the graph below. The percentage of positive cells during the both follicular and luteal phases of estrous cycle was significantly higher during the winter and spring season as compared to that during the summer and rainy seasons.



Graph: Seasonal variations in the ERα expression pattern in the oviduct of buffalo

Experiment 4. Immuno localization of estrogen receptor and progesterone receptor in uterus of buffalo during follicular and luteal phases of estrous cycle

A. Immuno localization of estrogen receptor alpha in uterus of buffalo

The uterus was histologically comprised of endometrium, myometrium and perimetrium. Endometrium is consisted of tunica mucosa and submucosa (Fig.1A). The surface epithelium of the tunica mucosa consisted of patches of simple columnar and pseudostratified epithelium (Fig.1B). Similar observations have been recorded by Pathak and Bansal (2011) in buffalo uterus by using transmission electron microscope. The sumucosa layer was richly endowed with endometrial glands.

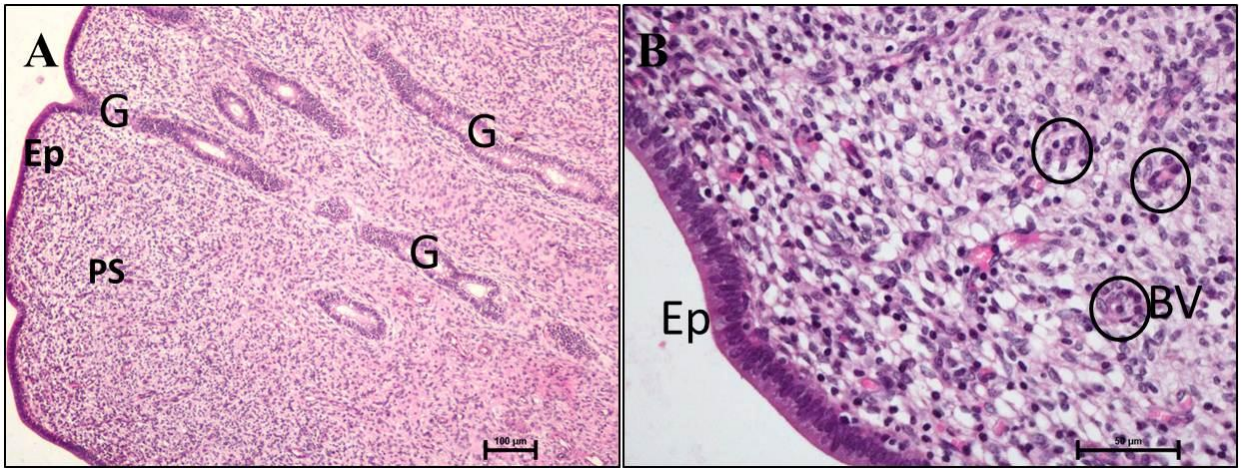


Fig.1: Uterine horn of buffalo showing, (**A)** Lining epithelium (Ep) of endometrium, propria submucosa (PS) and endometrial glands (G). H&E. x100; (**B)** Endometrium showing lining epithelium (Ep) with columnar cells, propria submucosa with numerous blood capillaries (BV). H&E. x400.

In the present investigation, ERα and PR was immuno localized using immunohistochemical technique with the use of specific antibodies against ERα and PR in tunica mucosa, submucosa, tunica muscularis and tunica serosa both during follicular and luteal phases of estrous cycle. The ERα was localized in luminal epithelium, glandular epithelium, stromal cells (Fig.2A and 2B) and myometrium (Fig.2C) and few cells in perimetrium. The endothelial cells lining the large arteries and capillaries present in the stratum vasculare of the myometrium were also strongly

positive for ERα (Fig. 2D). The immuno staining was observed in the nucleus of cells. However faint cytoplasmic reactions were also seen at places. In our study cytoplasmic staining was rarely observed.

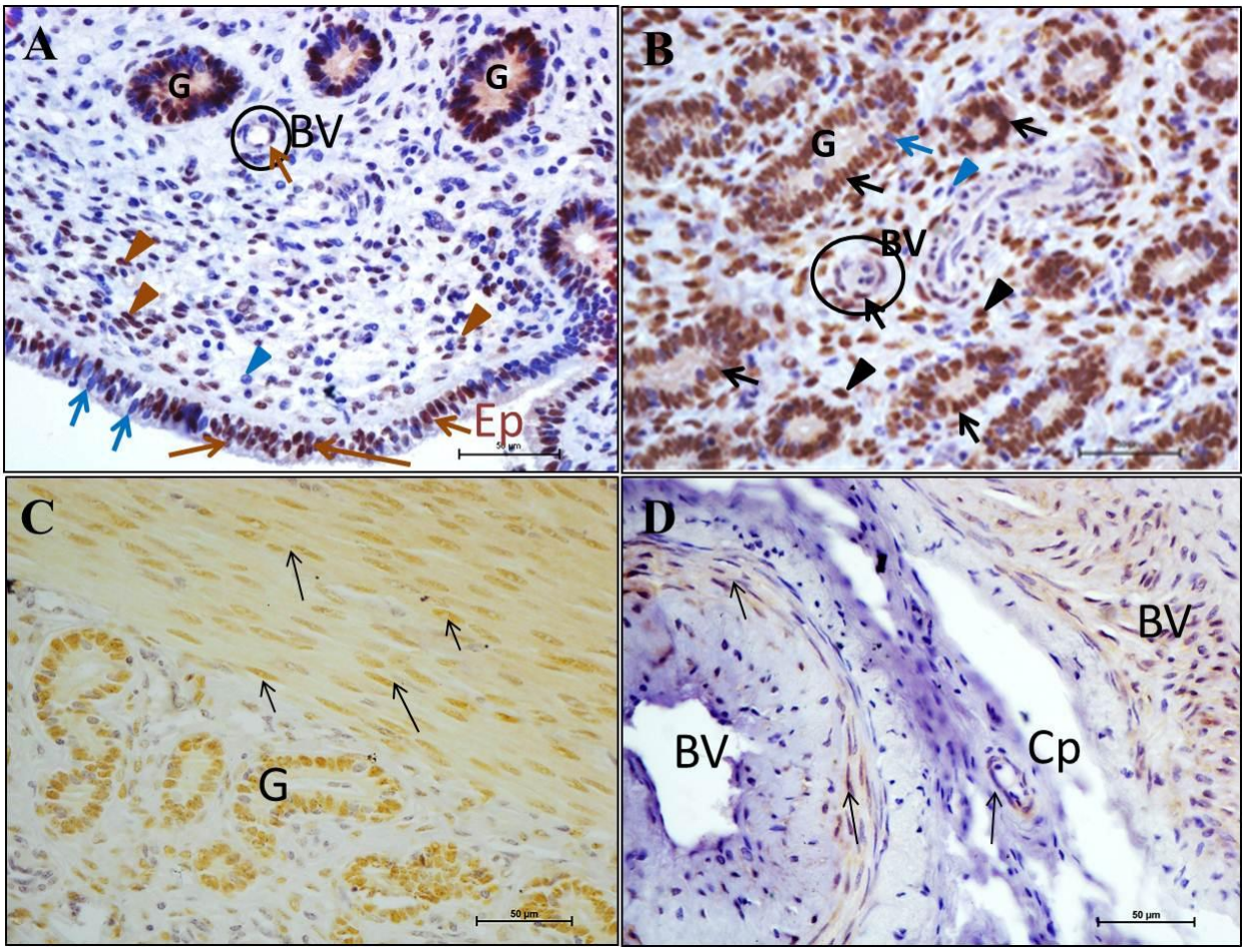


Fig. 2: Immunostaining of uterus during follicular phase with anti-ERα antibody showing nuclear reaction. (**A)** In the luminal epithelium (positive cells with brown arrows, negative cells with blue arrows), stromal cells in Propria submucosa (positive cells with brown arrow heads, negative cells with blue arrow heads), endothelial cells of blood vessels (arrow in circle with BV) and glandular epithelial cells (G); (**B)** in the stromal cells in Propria submucosa (positive cells with black arrow heads, negative cells with blue arrow heads), endothelial cells of blood vessels (arrow in circle with BV) and glandular epithelial cells (G); (**C)** In the smooth muscle cells in tunica muscularis (arrows) and glands in stratum compactum (G); (**D)** In endothelial cells in blood vessels (BV) and capillaries (Cp). Polymer HRP method. Original magnification x400

In the connective tissue stroma, the highest number of ER alpha positive cells was found during the follicular phase, which was significantly different compared to luteal phase (Fig. 2). Few endothelial cells and the cells located in peri-vascular area in the stroma were also positive were also positive for ERα (Fig.3A). No staining was observed in negative controls where the primary antibody was replaced with that of washing buffer (Fig. 3B). The presence intense reactions in different compartment of uterus indicated that all of the cell types were responsive to the estrogen hormone. The proliferation of the epithelial cells, stromal cells and glandular epithelium during the follicular phase might influenced by the estrogen hormone as indicated by presence of its receptors. The presence of ERα in the endothelial cells of the blood capillaries in endometrium and myometrium and larger vessels in the *stratum vasculare* layer of the myometrium inferred that the estrogen hormone was also controlling the active flow hormone during the estrous cycle.

The quantitative analysis based on the counting of percentage of ERα positive cells in different compartments of uterus of buffalo has been presented in figure 5. Variations were seen in the different tissue compartments of the uterus and during the different stages of the estrous cycle. Significantly higher number of ERα positive cells was observed in lamina epithelialis as compared to stromal cells and smooth muscle cells in myometrium. There was no significant difference in immuno reactivity between lamina epithelialis and lining epithelium of endometrial glands. Higher number of ERα positive cells was found during the follicular phase in all the compartments as compared to the luteal phase of estrous cycle. But there was significantly higher number of ERα cells in the lamina epithelialis and lining epithelium of endometrial glands during follicular phase as compared to the luteal phases of estrous cycle. There was no significant difference in the immuno reactivity in stromal cells and smooth muscle cells of myometrium during the phases of estrous cycle.

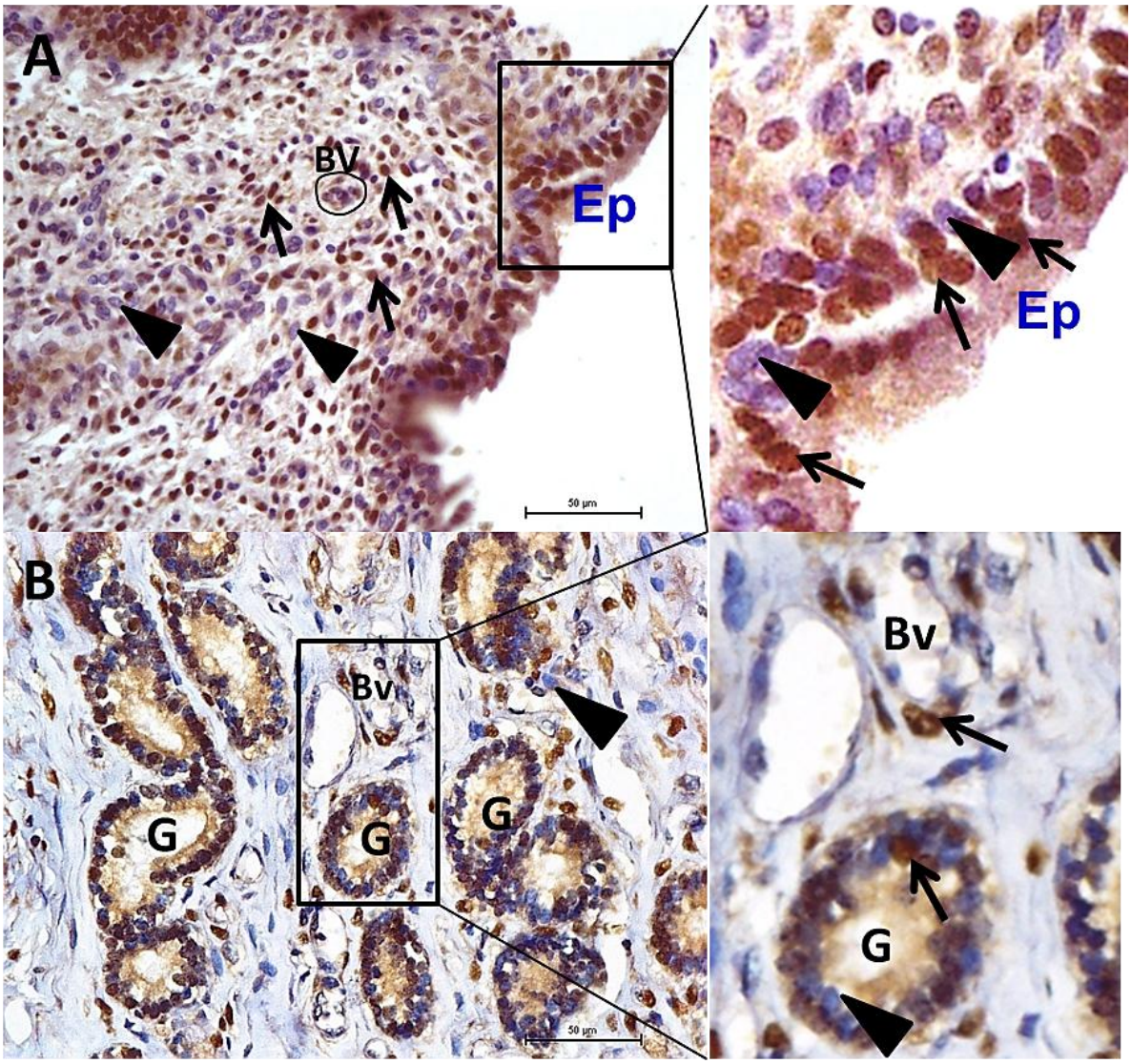


Fig. 3: Immunostaining of uterus of buffalo during luteal phase with anti-ERα antibody showing nuclear positive immunostaining (arrows) and negative reactions (arrow heads), (**A)** In the luminal epithelium (EP) and stromal cells and endothelial cells of blood vessels (BV) present in Propria submucosa; Magnified view of selected area showing distinct nuclear reaction in epithelial lining (Ep). (**B)** In the glandular epithelial cells (G), Endothelial cells of blood vessels (BV), Magnified view of selected area showing distinct nuclear reaction in glandular epithelium (G) and endothelial cells of blood vessels (BV). Polymer HRP method. Original magnification x400

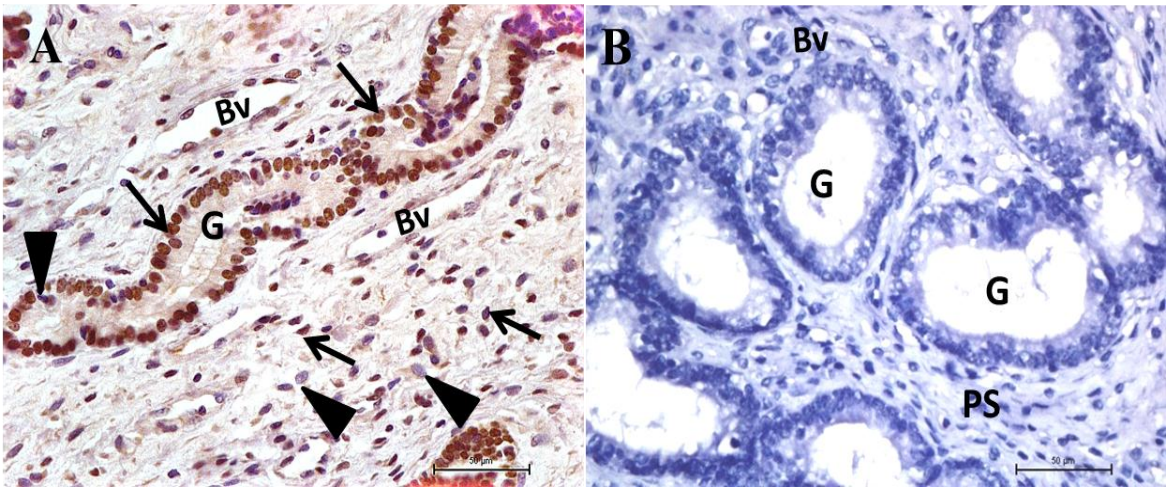


Fig.4: Immunostaining of uterus of buffalo during luteal phase with anti-ERα antibody showing, (**A)** Nuclear positive immunostaining (arrows) in the glandular epithelial cells (G), endothelial cells of blood vessels (Bv) and stromal cells, negative reactions (arrow heads); (**B)** No staining was observed in glands (G), propria submucosa (PS) and blood vessels (Bv) in negative controls. Polymer HRP method. Original magnification x400.

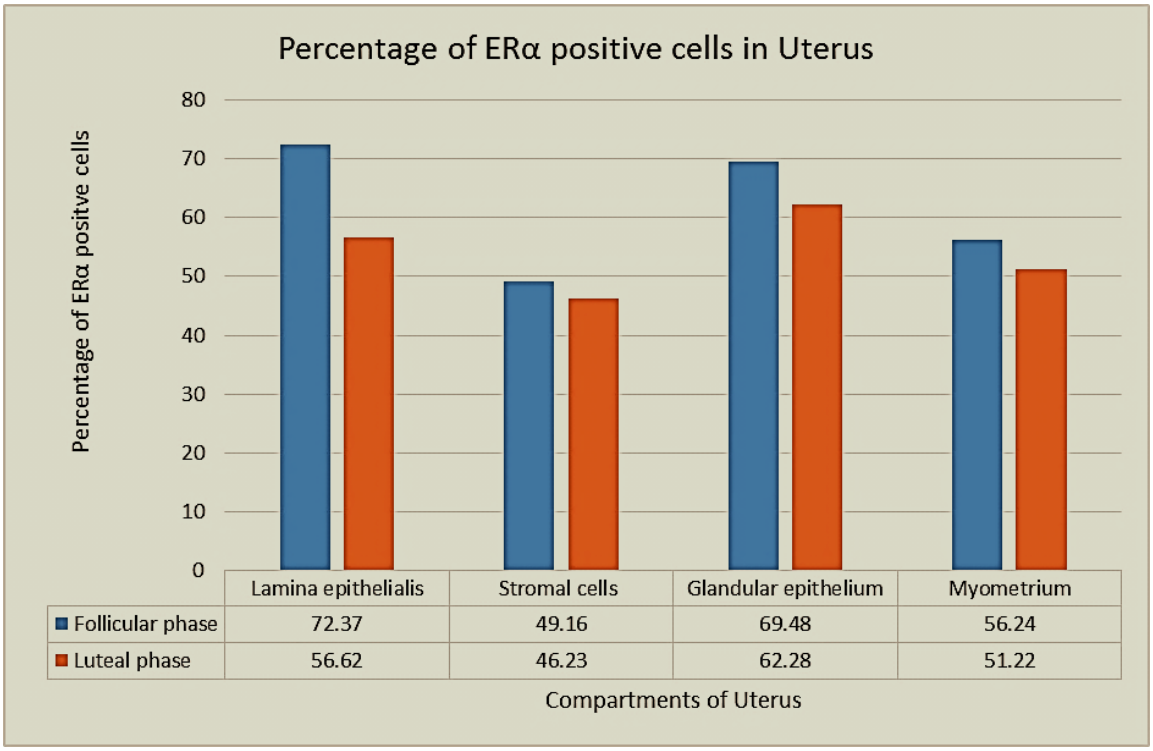


Fig. 5: Quantitative analysis of PR positive cells in different compartments of Uterus

B. Immuno localization of progesterone receptor in uterus of buffalo

The PR was localized in luminal epithelium (Fig. 5A), glandular epithelium (Fig. 5B), stromal cells and endothelial cells of blood vessels present in the propria sub mucosa (Fig.5A and 4B) and myometrium and few cells in perimetrium of buffalo uterus during the follicular phase of estrous cycle. The immuno staining was observed both in the nuclei and cytoplasm of cells, however the reactivity in nuclei was strong while in the cytoplasm was weak to moderate as seen as faint staining in the cytoplasm. During the luteal phase of the estorus cycle, intense nuclear reaction was observed in the lamina epithelialis and stromal cells in the propria submucosa (Fig.5C). Intense nuclear reaction was also observed in the lining epithelium of endometrial glands (Fig. 5D). The lining epithelium of endometrial glands in both the zones viz; stratum spongiosum and stratum compactum were immuno positive for PR. In lamina epithelialis and glandular epithelium, immunoreactivity was higher in follicular phase as compared with luteal phase of oestrous cycle. Variations were seen in the different tissue compartments of the uterus and during the different stages of the estrous cycle. Few endothelial cells and the cells located in peri-vascular area in the stroma were also positive were also positive for PR. No staining was observed in negative controls.

It was concluded from the present study that the ERα was localized in luminal epithelium, glandular epithelium, stromal cells and myometrium and few cells in perimetrium. The immuno staining was observed in the nucleus of cells. In lamina epithelialis immunoreactivity was higher in follicular phase as compared with luteal phase of estrous cycle. The PR was localized in luminal epithelium, glandular epithelium, stromal cells and endothelial cells of blood vessels present in the propria sub mucosa and myometrium and few cells in perimetrium of buffalo uterus during the follicular and luteal phases of estrous cycle. Thus estrogen and progesterone play a major role in controlling the physiology of uterus by acting through their respective nuclear receptors.

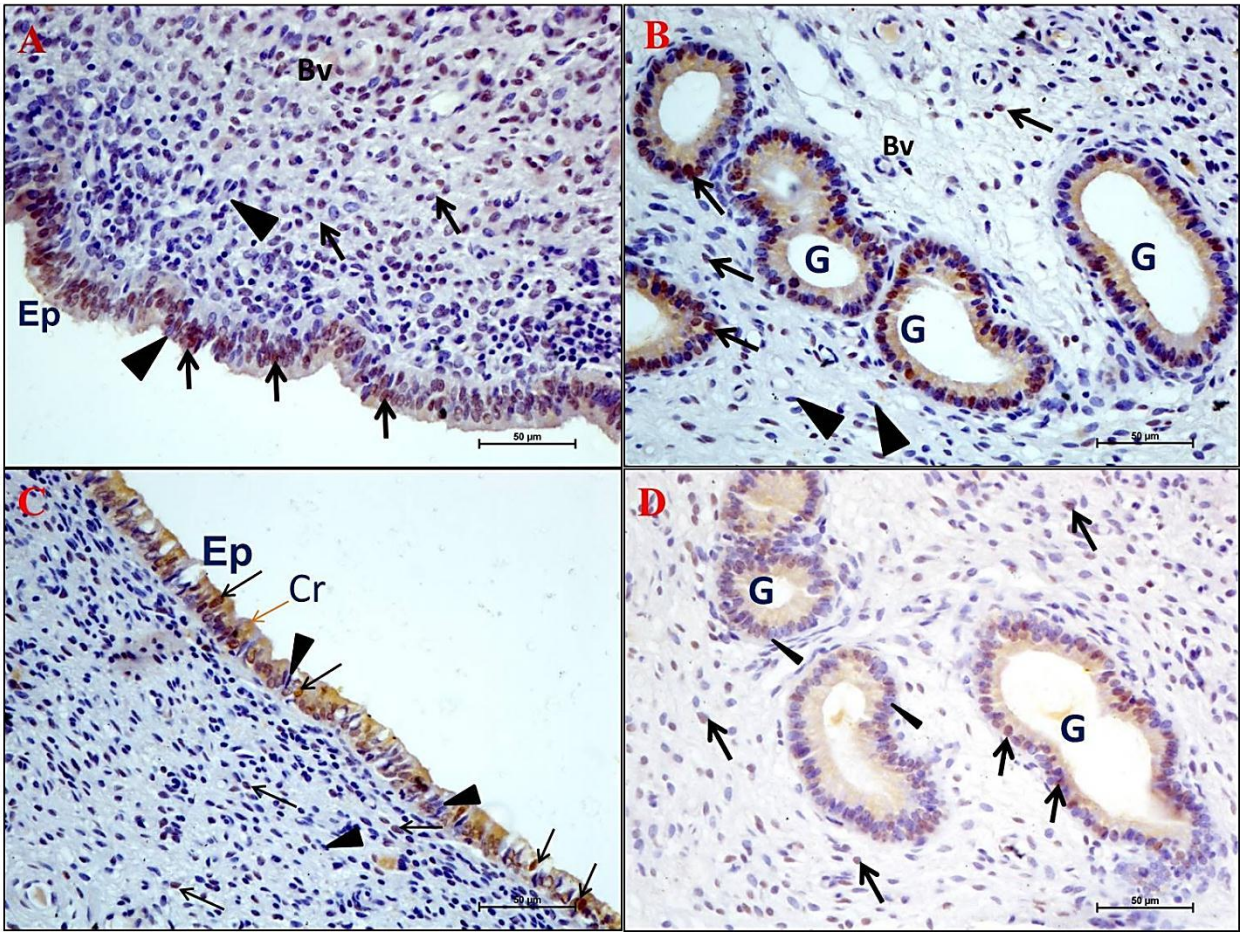


Fig. 6: Immunostaining of uterus of buffalo with anti-PR antibody showing nuclear positive immunostaining (arrows) and negative reactions (arrow heads), (**A)** in the luminal epithelium (EP) and stromal cells and endothelial cells of blood vessels (BV) present in Propria submucosa during follicular phase; (**B)** in the glandular epithelial cells (G), Endothelial cells of blood vessels (BV) and stromal cells during follicular phase; (**C)** in the luminal epithelium (EP) and stromal cells and glandular epithelial cells present in Propria submucosa during luteal phase; (**D)** in the glandular epithelial cells (G) and stromal cells during luteal phase; Polymer HRP method. Original magnification x400.

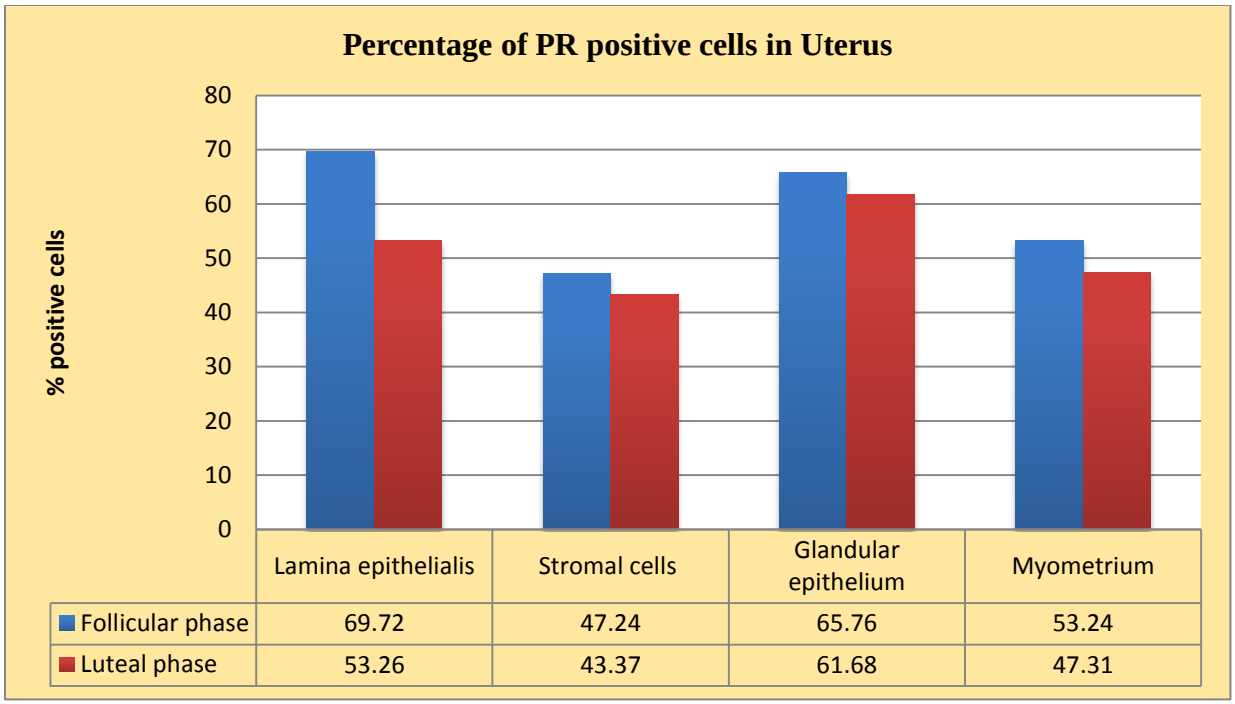


Fig.7: Quantitative analysis of PR positive cells in different compartments of Uterus

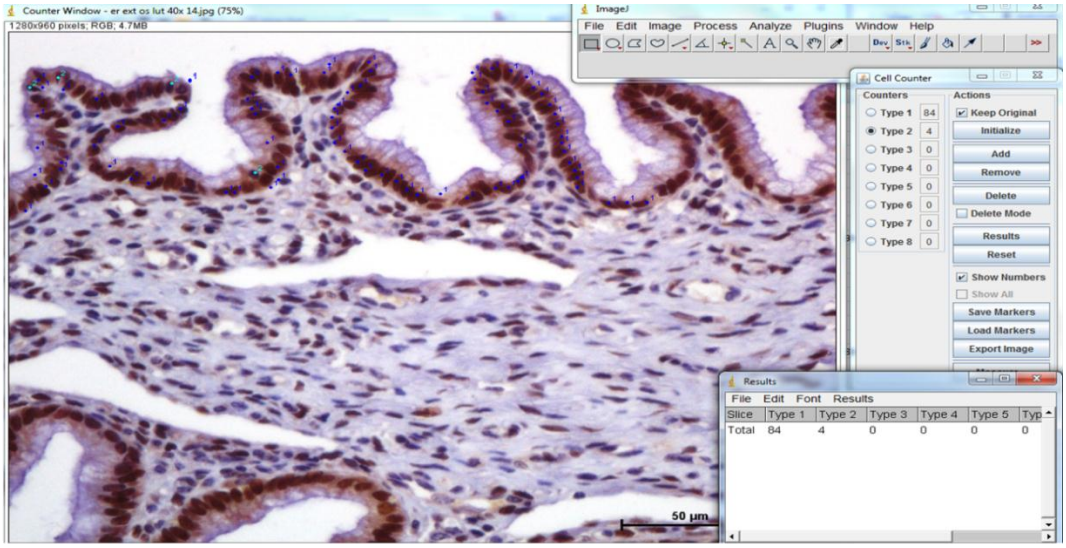
The quantitative analysis based on the counting of percentage of PR positive cells in different compartments of uterus of buffalo has been presented in figure 7. Significantly higher number of PR positive cells was observed in lamina epithelialis as compared to stromal cells and smooth muscle cells in myometrium ($P < 0.05$). There was no significant difference in immuno reactivity between lamina epithelialis and lining epithelium of endometrial glands. Significantly higher number of PR positive cells was observed in the lamina epithelialis during follicular phase as compared to the luteal phases of estrous cycle ($P < 0.05$). There was no significant difference in the immuno reactivity in stromal cells, lining epithelium of endometrial glands and smooth muscle cells of myometrium during the phases of estrous cycle.

Experiment 4. Immuno localization of estrogen receptor and progesterone receptor in cervix uteri of buffalo during follicular and luteal phases of estrous cycle.

A. Immuno localization of estrogen receptor in cervix uteri

Cervix Located between the vagina and the uterus and is designed to restrict access to the uterus.Walls of the cervix are very thick and the body of the cervix contains three or four rings called annular folds. The cervix is hormone-dependent tissue. The effect of estrogen and progesterone upon the cervix is dependent upon the availability of hormone receptors in different cell types. Therefore, the distribution and amount of receptors is as critical as the amount of hormone in predicting hormone action. Information on the distribution of steroid hormone receptors in the various cell types in cervix is useful to better analyze the changes occurring in normal cervix during the estrous cycle and also to understand why different cell types react differently on the same hormonal stimuli.

The present investigation was aimed at determination of distribution of estrogen and progesterone receptors in cervix uteri of cyclic Indian buffalo during follicular and luteal phases of estrous cycle.



% of positive cells were statistically analyzed to find out difference of expression during follicular and luteal phases of estrous cycle

ERα immunoreactions occurred in the nuclei of epithelial cells, connective tissue cells and smooth muscle cells of the cervix during both the follicular and luteal phases.Nuclear staining for ERα and PR was observed in the epithelial cells of the surface epithelium, stromal cells and

smooth muscle cells. In the cervix, ERα immunoreactivity was more intense in the epithelial, cervical glands and smooth muscle cells during the follicular phase. These results indicated that the frequency and intensity of ERα immunoreactivity in the cervix of buffaloes varied according to the cervical cell types and the phases of the sexual cycle.

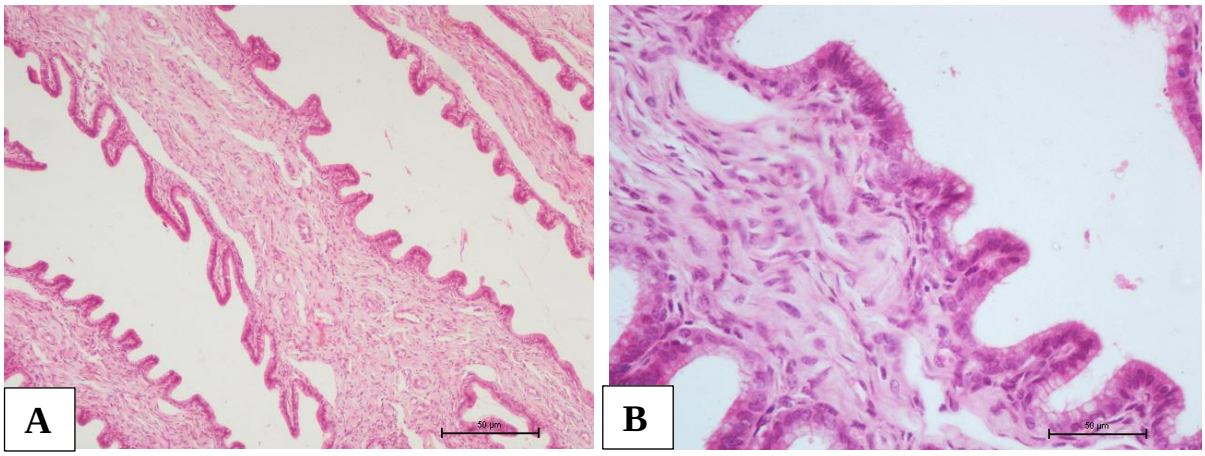


Fig.4. Paraffin section of cervix uteri of buffalo showing: **A**. mucosal folds with primary and secondary branches. x100; **B.** Mucosa lined with columnar cells x400. Haematoxylin and eosin stain.

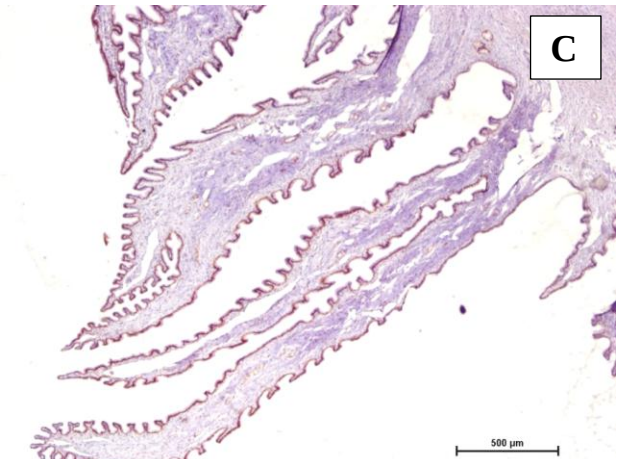


Fig.4C. Immunostaining of cervix uteri of buffalo with anti-ERα antibody. Nuclear positive immunostaining epithelial cells and stromal cells in the Propria submucosa. Polymer HRP method. Original magnification x100

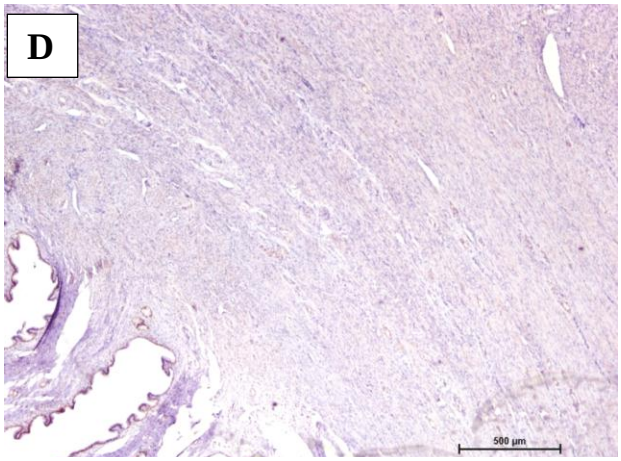


Fig.4D. Immunostaining of cervix uteri of buffalo with anti-ERα antibody. Nuclear positive immunostaining in tunica muscularis. Polymer HRP method. Original magnification x100

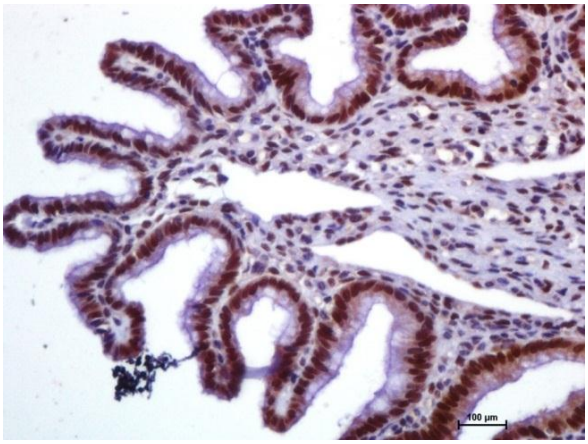


Fig.4E. Immunostaining of cervix uteri of buffalo with anti-ERα antibody. Nuclear positive immunostaining epithelial cells and stromal cells in the Propria submucosa. Polymer HRP method. Original magnification x400

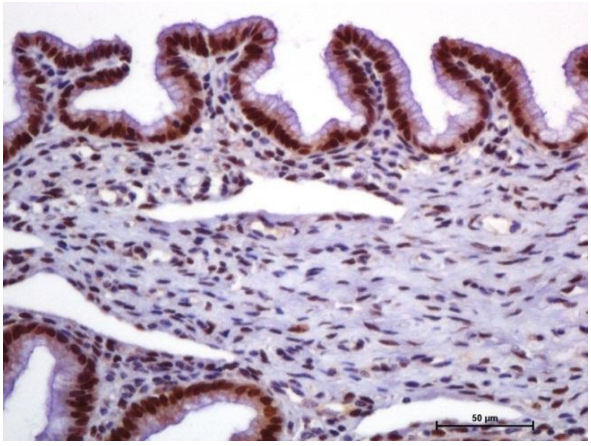


Fig.4F. Immunostaining of cervix uteri of buffalo with anti-ERα antibody. Nuclear positive immunostaining epithelial cells and stromal cells in the Propria submucosa. Polymer HRP method. Original magnification x400

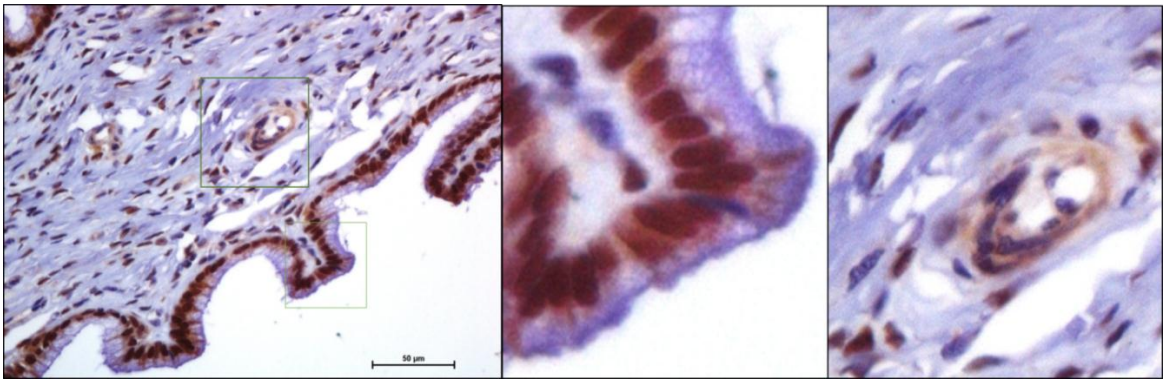


Fig.4G. Immunostaining of cervix uteri of buffalo with anti-ERα antibody. Nuclear positive immunostaining in epithelial cells, endothelial cells of blood vessels and stromal cells in the Propria submucosa. (a)Magnified view of immune reaction in lamina epithelialis and (b) magnified view of immune reaction in endothelial cells and stromal cells. Polymer HRP method. Original magnification x400

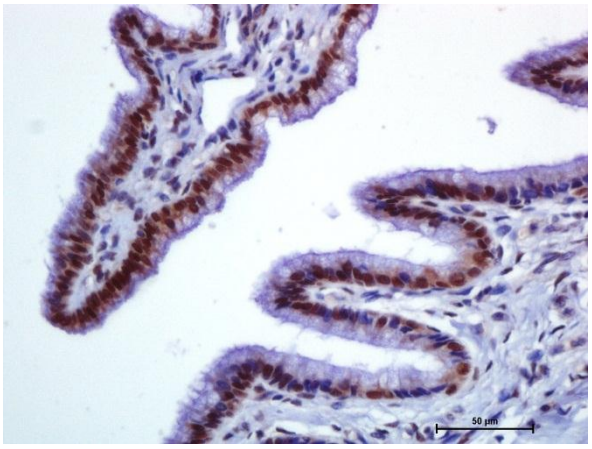


Fig.4E. Immunostaining of Internal os of cervix uteri in follicular phase of buffalo with anti-ERα antibody. Nuclear positive immunostaining epithelial cells and stromal cells in the Propria submucosa. Polymer HRP method. Original magnification x400

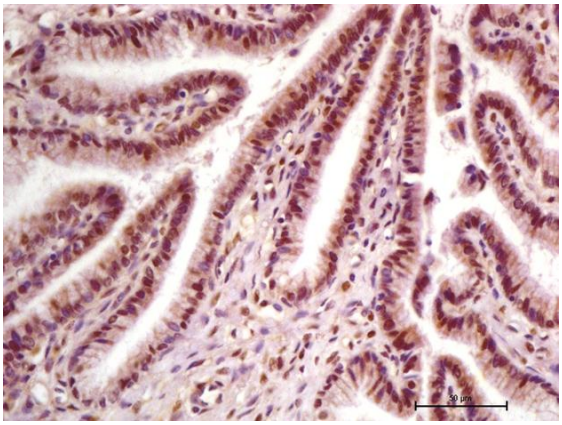


Fig.4E. Immunostaining of Internal os of cervix uteri in follicular phase of buffalo with anti-ERα antibody. Nuclear positive immunostaining epithelial cells and stromal cells in the Propria submucosa. Polymer HRP method. Original magnification x400

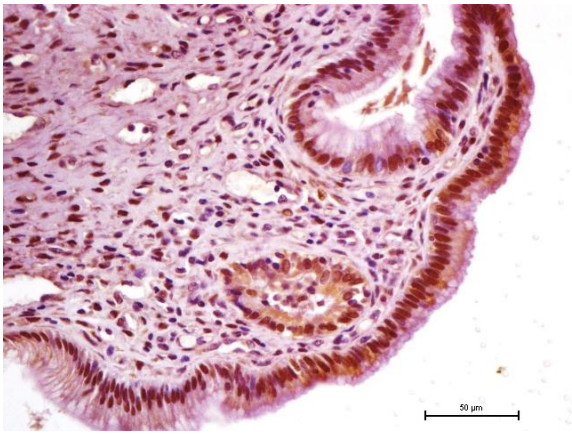


Fig.4E. Immunostaining of external os of cervix uteri in follicular phase of buffalo with anti-ERα antibody. Nuclear positive immunostaining epithelial cells and stromal cells in the Propria submucosa. Polymer HRP method. Original magnification x400

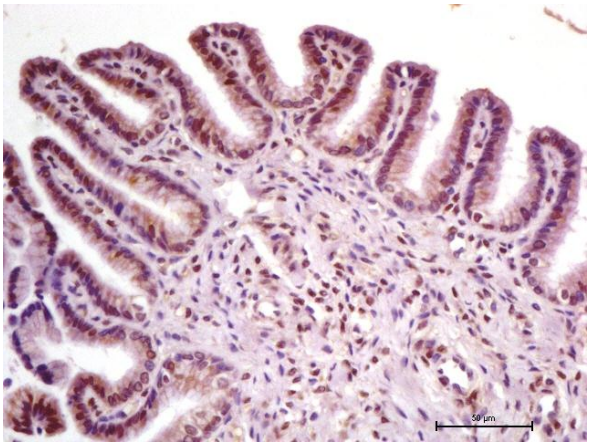


Fig.4E. Immunostaining of external os of cervix uteri in luteal phase of buffalo with anti-ERα antibody. Nuclear positive immunostaining epithelial cells and stromal cells in the Propria submucosa. Polymer HRP method. Original magnification x400

B. Immuno localization of estrogen receptor in cervix uteri

The present investigation aimed at determination of distribution of progesterone receptors in cervix uteri of cyclic Indian buffalo during follicular and luteal phases of estrous cycle. The tissue distribution of PR was determined by immunohistochemical technique using one step polymer horseradish peroxidase staining system. Nuclear staining for PR was observed in the epithelial cells of the surface epithelium, stromal cells and smooth muscle cells. The lining epithelium of cervical glands showed intense positive nuclear reaction for progesterone receptor. The lining epithelium of cervical glands showed intense positive nuclear reaction for progesterone receptor. These results indicated that the frequency and intensity of PR immunoreactivity in the cervix of buffaloes varied according to the cervical cell types and the phases of the sexual cycle.

Fig.4A. Immunostaining of cervix uteri of buffalo with anti-PR antibody. Nuclear positive immunostaining in the luminal and glandular epithelial cells and stromal cells. Polymer HRP method. Original magnification x400

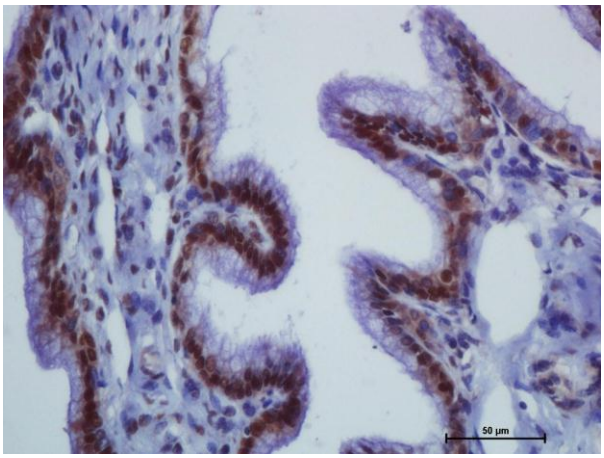


Fig.4A. Immunostaining of cervix uteri of buffalo with anti-PR antibody. Nuclear positive immunostaining in the luminal and glandular epithelial cells and stromal cells. Polymer HRP method. Original magnification x400

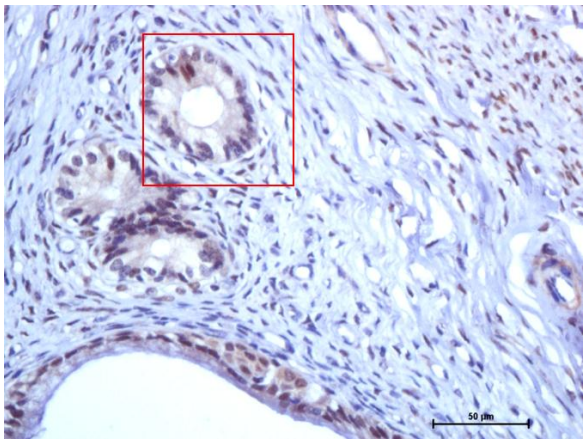


Fig.4A. Immunostaining of cervix uteri of buffalo with anti-PR antibody. Nuclear positive immunostaining in the luminal and glandular epithelial cells and stromal cells. Polymer HRP method. Original magnification x400

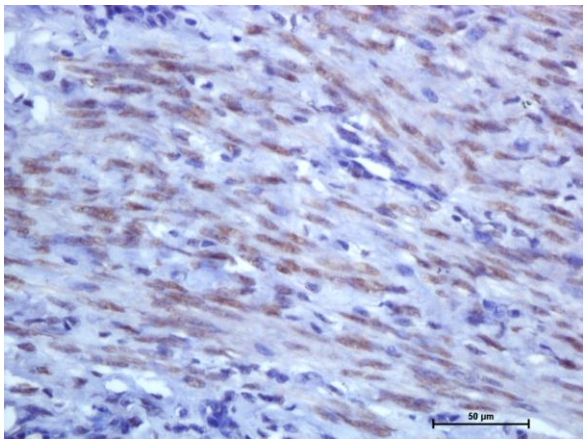


Fig.4B. Immunostaining of cervix uteri of buffalo with anti-PR antibody. Nuclear positive immunostaining in the luminal and glandular epithelial cells and stromal cells. Polymer HRP method. Original magnification x400

- No immunostaining was observed in any compartment of cervix uteri when the primary antibody was replaced by antibody diluent or buffer which served as negative control.

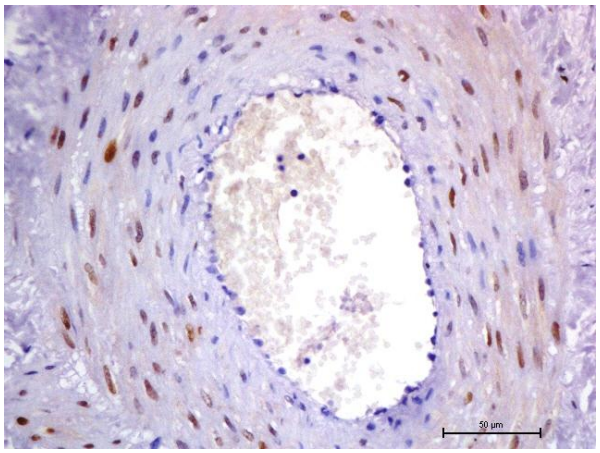


Fig.4C. Immunostaining of cervix uteri of buffalo with anti-PR antibody. Nuclear positive immunostaining in the muscle cells of muscular arteries. Polymer HRP method. Original magnification x400

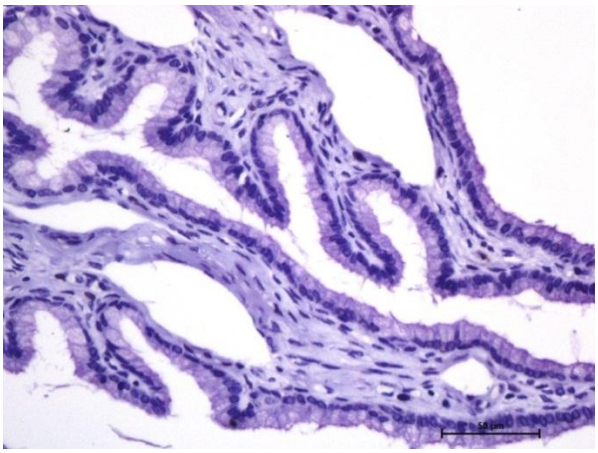


Fig.4C. Negative control where primary antibody was replaced with washing buffer. Polymer HRP method. Original magnification x400

Possible co-localization of ER and PR in Cervix uteri

In serial sectioning, subsequent sections were stained for estrogen and progesterone receptors respectively. It was found that same cells in the subsequent sections were stained for estrogen and progesterone receptors (Fig. 4C and). Thus it could be concluded that there is a possible colocalization of these receptors in same cells. Thus both the receptors might be present in most the cells and would have been responding to the circulating hormonal levels of estrogen and progesterone.

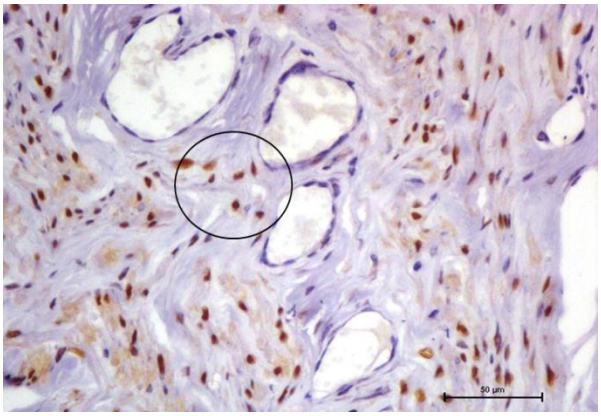


Fig.4C. Immunostaining of cervix uteri of buffalo with anti-PR antibody. Nuclear positive immunostaining in the muscle cells of muscular arteries. Polymer HRP method. Original magnification x400

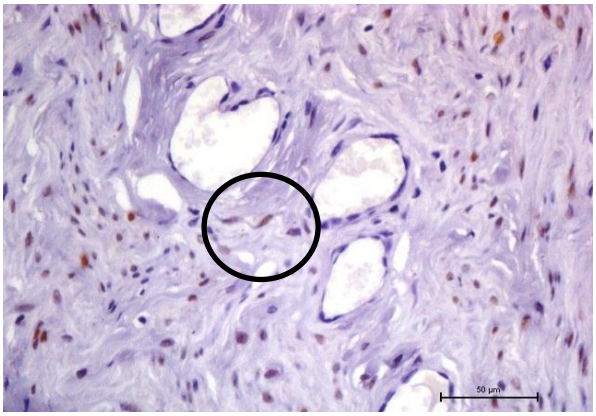


Fig.4C. Immunostaining of cervix uteri of buffalo with anti-PR antibody. Nuclear positive immunostaining in the muscle cells of muscular arteries. Polymer HRP method. Original magnification x400

D. Immuno localization of PCNA and Ki 67 in cervix uteri of buffalo

Immuno localization of Ki67 and PCNA was used to determine the relation of proliferation potential of estrogen hormone. The results indicated that the status of cervix uteri using expression of proliferation marker PCNA and Ki67 was well correlated to the ERα expression in cervix uteri of buffalo.

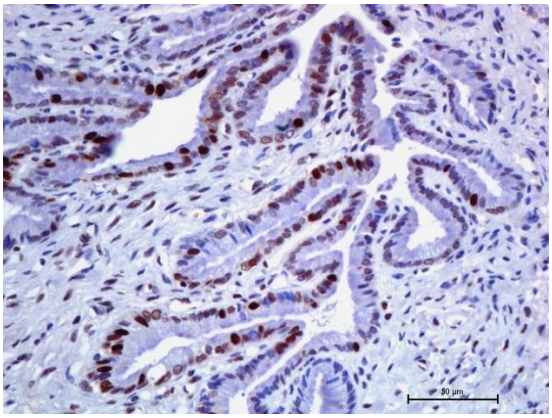


Fig.4C. Immunostaining of cervix uteri of buffalo with anti-PCNA antibody. Nuclear positive immunostaining in the lamina epithelialis and stromal cells in Propria submucosa. Polymer HRP method. Original magnification x400

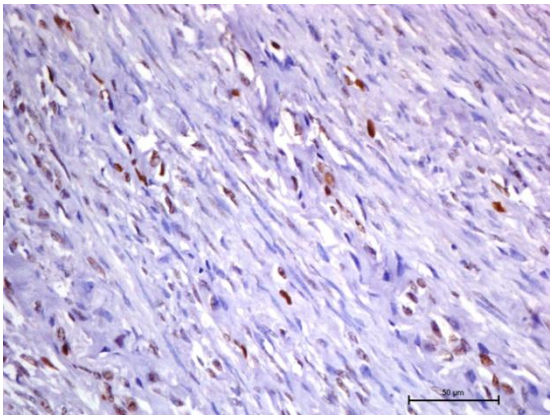


Fig.4C. Immunostaining of cervix uteri of buffalo with anti-PR antibody. Nuclear positive immunostaining in the muscle cells of muscular arteries. Polymer HRP method. Original magnification x400

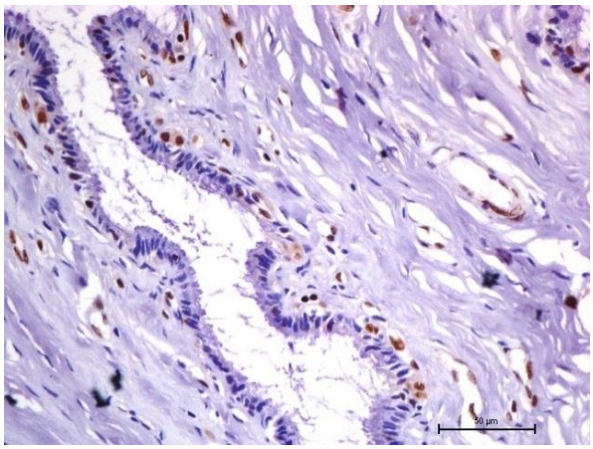


Fig.4C. Immunostaining of cervix uteri of buffalo with anti-PR antibody. Nuclear positive immunostaining in the muscle cells of muscular arteries. Polymer HRP method. Original

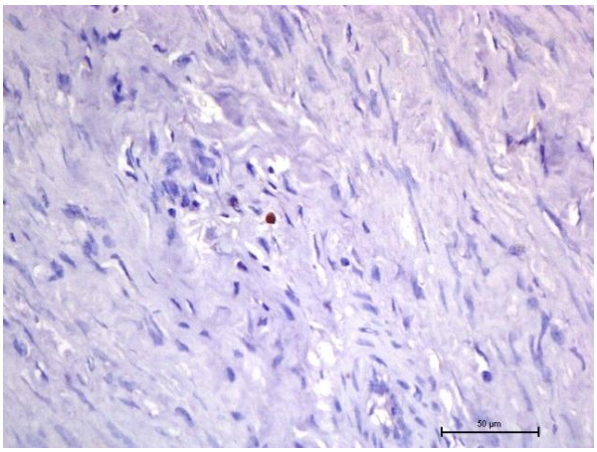


Fig.4C. Immunostaining of cervix uteri of buffalo with anti-PR antibody. Nuclear positive immunostaining in the muscle cells of muscular arteries. Polymer HRP method. Original magnification x400

Hormone estimation

Hormone level	Follicular phase	Luteal phase
Estradiol	28.82±1.13 ng/ml	13.13±1.18 ng/ml
Progesterone	0.13±0.42 pg/ml	2.63±0.62 pg/ml

The average concentrations of plasma estradiol during follicular and luteal phase of the animals under the study were 28.82±1.13 pg/ml and 13.13±1.18 pg/ml respectively. The average concentration of plasma progesterone during follicular and luteal phase of the animals under the study was 0.13±0.42 ng/ml and 2.63±0.62 ng/ml respectively. The data on receptor and hormone analysis showed that the ER and PR expression was higher during the estradiol dominance and lower during the progesterone dominance.

References

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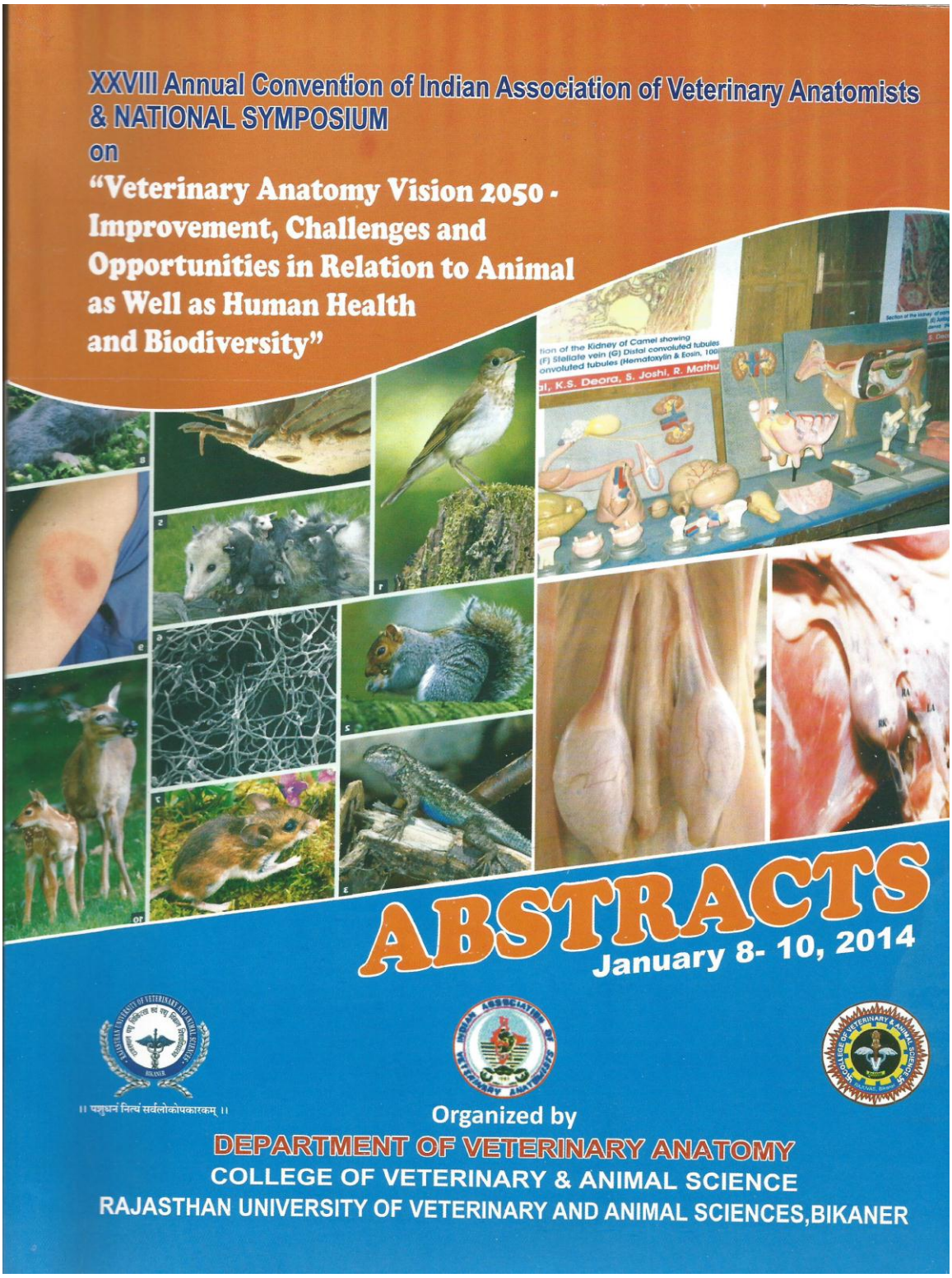

XXVIII Annual Convention of Indian Association of Veterinary Anatomists
& NATIONAL SYMPOSIUM
on
"Veterinary Anatomy Vision 2050 -
Improvement, Challenges and
Opportunities in Relation to Animal
as Well as Human Health
and Biodiversity"
ABSTRACTS
January 8- 10, 2014
॥ पशुधनं नित्यं सर्वलोकोपकारकम् ॥
Organized by
DEPARTMENT OF VETERINARY ANATOMY
COLLEGE OF VETERINARY & ANIMAL SCIENCE
RAJASTHAN UNIVERSITY OF VETERINARY AND ANIMAL SCIENCES,BIKANER

DAKO A/S, Denmark) to identify alveolar type 2 cells. The immunopostive alveolar type 2 cells were counted manually per 0.08mm² area at 100X under oil immersion. The expression profile of TNFá mRNA and immunopostive alveolar type 2 cells were compared among control and treatment groups. It is the preliminary data on the expression profile of TNFá mRNA and alveolar type 2 cells after single and multiple exposures to poultry barn air.

Immunolocalization of Progesterone Receptor in Oviduct of Buffalo

DevendraPathak, NeelamBansal, Kuldip Gupta and SPS Ghuman

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana

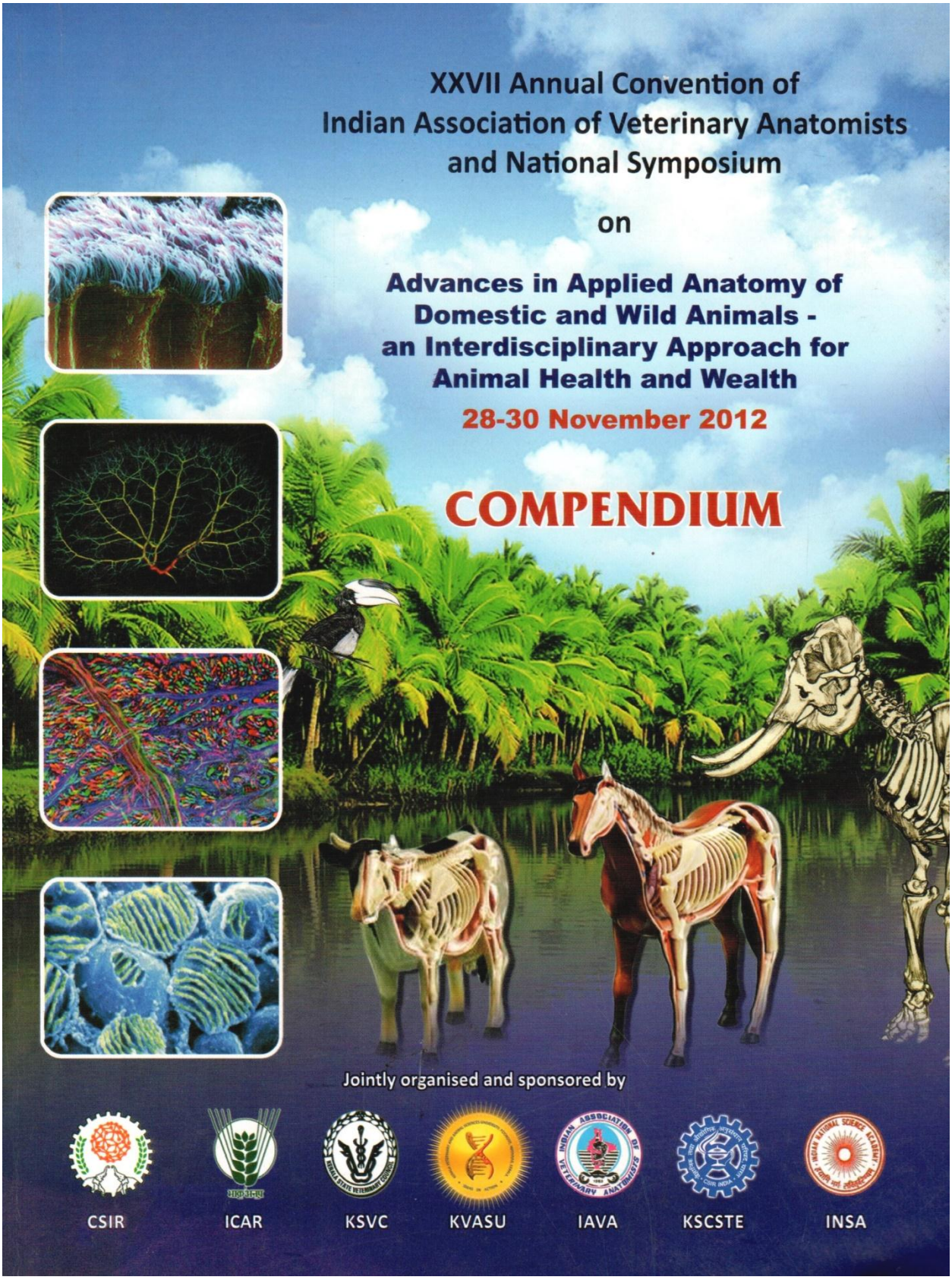
The steroid hormone, progesterone, is a key modulator of normal reproductive functions of animals. The present investigation aimed at determination of distribution of progesterone receptor in different segments of oviduct of buffalo during follicular and luteal phases of estrous cycle. Oviducts from twelve buffaloes (six each during follicular and luteal phases) were collected from slaughter house. Blood samples were also collected from the animalsbefore slaughter to estimate levels of estrogen and progesterone hormones. The tissue distribution of progesterone receptor was determined by immunohistocheemical technique using one step polymer HRPO staining system. The progesterone receptors were localized in lamina epithelialis, propriasubmucosa, tunica muscularis and tunica serosa.The maximum localization was observed in lamina epithelialis, where both ciliated and secretory cell types were positive for progesterone receptors. Percentage of positive cells varied during the follicular and luteal phases of estrous cycle. The lining epithelium of oviductal glands formed due to in folding of mucosal folds or joining of the two mucosal adjacent folds were also intensely positive for progesterone receptor.Noimmunostaining was observed in any compartment of oviduct when the primary antibody was replaced by antibody diluent or buffer and it served as negative control.

Immunolocalization of Estrogen Receptor Alpha in Uterus of Buffalo

DevendraPathak, NeelamBansal and Kuldip Gupta

Guru AngadDev Veterinary and Animal Sciences University, Ludhiana

In order to understand physiological changes during the different stages of the oestrous cycle, immunohistochemistry was used in the present study to investigate the distribution of oestrogen receptor alpha (ERá) in the buffalo uterus during follicular and luteal phases of oestrouscycle.Uteri from twelve buffaloes (six each during follicular and luteal phases) were collected from slaughter house. The tissue distribution of estrogen receptor alpha was examined using immunohistochemical technique (one step super sensitive polymer based horse radish peroxidases). In the uterus, intense nuclear ER alpha staining occurred in theluminal and glandular epithelial cells, stromal cells, muscle cells and few cells in perimetrium. The immuno staining was observed in the nucleus of cells. In lamina epithelialisimmunoreactivity was higher in follicular phase as compared with luteal phase of oestrous cycle. In the myometrium, the highest levels of staining of ERá positive cells were found during the follicular phase. Variations were seen in the different tissue compartments of the uterus and during the different stages of the oestrous cycle. In the connective tissue stroma, the highest number of ERá positive cells was found during the follicular phase, which was significantly different compared to luteal phase. Few endothelial cells were also positive and cells located in perivascular area in the stroma were also positive. No immunostaining was observed in any compartment of uterus when the primary antibody was replaced by antibody diluent or buffer and it served as negative control.


XXVII Annual Convention of
Indian Association of Veterinary Anatomists
and National Symposium
on
Advances in Applied Anatomy of
Domestic and Wild Animals -
an Interdisciplinary Approach for
Animal Health and Wealth
28-30 November 2012
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INSA

7.1 Immuno Localization of Estrogen Receptor in Oviduct of Buffalo during Follicular and Luteal Phases of Estrous Cycle

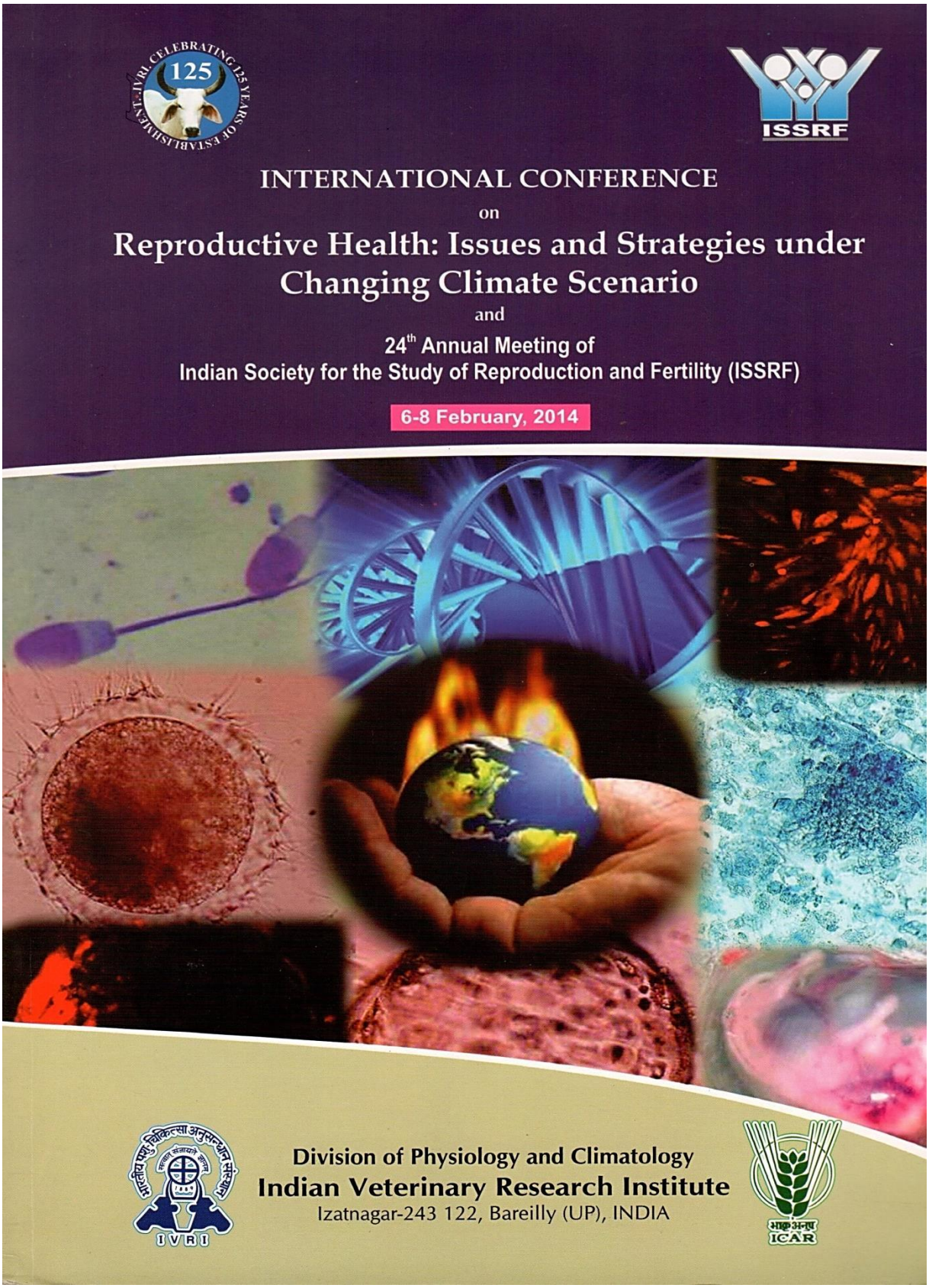
Devendra Pathak, Neelam Bansal, Kuldip Gupta and Ghuman, S. P. S.
Department of Veterinary Anatomy, College of Veterinary Science,
Guru Angad Dev Veterinary and Animal Sciences University,
Ludhiana, Punjab – 141004.

With immnuohistochemical staining oviducts from twelve buffaloes were screened for the presence of estrogen receptor alpha (ERα) during follicular and luteal phase of estrous cycle. ERs were detected on paraffin sections using one step poly HRPO staining technique and the reactivity was scored semi-quantitatively. The positive immunostaining was detected in the nuclei of the cells. ERα was observed in infundibulum, ampulla, and isthmus of buffalo oviduct. The nuclei of epithelial, stromal, and smooth muscle cells showed nuclear localization of estrogen receptors. Comparable staining intensities and distributions of estrogen receptor localization were detected in different portions of the oviduct. The serosa, endothelial cells of blood vessels and the connective tissues in muscularis showed weak or no staining for estrogen receptor. No significant variation was observed in the staining intensity in the muscle layer. Overall, the intensity of staining for ER was strong in oviduct during follicular phase, while it was moderate to strong during the luteal phase of the estrous cycle. The results indicated that oviductal ERα in buffalo varied according to the phase of the ovarian activity.

7.2 Immunohistochemistry for RANK, RANKL and OPG in the Normal and Inflamed Lungs of Buffalo Calves

Sethi, R. S., Nidhi, R. S., Brar and Baljit Singh
School of Animal Biotechnology,
Guru Angad Dev Veterinary and Animal Sciences University,
Ludhiana, Punjab- 141004.

Receptor activator of nuclear factor kappa B (RANK), its ligand (RANKL) and osteoprotegerin (OPG) system are the dominant final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ISSRF
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on
Reproductive Health: Issues and Strategies under Changing Climate Scenario
and
24th Annual Meeting of
Indian Society for the Study of Reproduction and Fertility (ISSRF)
6-8 February, 2014
Division of Physiology and Climatology
Indian Veterinary Research Institute
Izatnagar-243 122, Bareilly (UP), INDIA
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Oral Lectures

OL-10

Immunolocalization of Estrogen Receptor Alpha and Progesterone Receptor in *Cervix Uteri* of Indian Buffalo

Devendra Pathak, Neelam Bansal and Kuldip Gupta

Department of Veterinary Anatomy, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004
Email: drdevendra@gmail.com

The uterine cervix is a well known target tissue for sex steroids as it contains specific protein receptors (ERα and PR) for estrogen and progesterone. The present investigation aimed at determination of distribution of estrogen and progesterone receptors in cervix uteri of cyclic Indian buffalo during follicular and luteal phases of estrous cycle. The estrous cycle stage of 12 buffaloes was assessed by gross and histological appearance of ovaries. The tissue distribution of ER alpha and PR was determined by immunohistochemical technique using one step polymer horseradish peroxidase staining system. Nuclear staining for ERα and PR was observed in the epithelial cells of the surface epithelium, stromal cells and smooth muscle cells. In the cervix, ERα immunoreactivity was more intense in the epithelial, cervical glands and smooth muscle cells during the follicular phase. The lining epithelium of cervical glands showed intense positive nuclear reaction for progesterone receptor. These results indicated that the frequency and intensity of ERα and PR immunoreactivity in the cervix of buffaloes varied according to the cervical cell types and the phases of the sexual cycle. Preliminary results indicated that the status of cervix uteri using expression of proliferation marker Ki67 was well correlated to the ERα expression in cervix uteri of buffalo. No immunostaining was observed in any compartment of cervix uteri when the primary antibody was replaced by antibody diluent or buffer which served as negative control.

ABSTRACT-CUM-SOUVENIR BOOK

Dr. S.S. Guraya Memorial Seminar

on

Recent Advances in Animal Reproduction

October 12, 2016

Organized by

Department of Zoology
Punjab Agricultural University, Ludhiana
in association with Zoological Society

Supported by

Punjab State Council of Science & Technology, Chandigarh

Science and Engineering Research Board
New Delhi

Indian Council of Medical Research, New Delhi

Dr. S.S. Guraya Memorial Seminar on Recent Advances in Animal Reproduction (12th October, 2016)

B16

Histomorphochemical features and progesterone receptor expression in atretic follicles in Indian buffalo

Devendra Pathak* and Neelam Bansal

Department of Veterinary Anatomy, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, Punjab
**Email: drdevendra@gmail.com*

The present study was conducted on ovaries of buffaloes (n = 48) collected from different slaughter houses and TVCC, GADVASU, Ludhiana. Ovaries were processed for the histo-morphochemical and immuno-histochemical studies. In all reproductive stages, ovary presented follicle in different stages of development. Some of the follicles of each type showed degenerative changes and led to atresia. The atretic follicle at primordial and primary follicle level was seen as collapsed structure. The ooplasm was minimal or totally absent. In the tertiary follicle two distinct types of atresia was observed. In the first type, the degeneration of membrana granulosa cells, theca cells occurred and lead to fluid filled cavity. The earliest sign of the atresia was presence of few granulosa cells with pyknotic nucleus and separation of the membrana granulosa cells from the basal lamina. At later stage, large number of granulosa cells and theca cells showed signs of apoptosis. In the second type of atresia the basal lamina folded in and growth of the stromal cells occurred which filled the whole follicular cavity and is referred as obliterative atresia. Atretic follicles showed moderate to strong PAS positive reaction in the basal lamina. Tertiary atretic follicle showed strong PAS positive material in ooplasm. In the obliterative atretic follicle strong alcinophilic and PAS positive secretion in centre and periphery of follicle was observed. In the cystic atresia the central fluid in the antrum consisted of alcinophilic material while in the periphery both PAS positive and alcinophilic membrane was observed. In the scar of the atretic follicle a thin basal line presented the PAS positive reaction. In the obliterative atretic follicles the invading stromal cells were highly positive for progesterone receptor indicating that atresia is a secretory event and corroborating with histochemical results.

Keywords: Atretic follicles; Histomorphochemical features; Indian buffalo; Progesterone receptor expression

INDIAN ASSOCIATION OF VETERINARY ANATOMISTS

XXVII ANNUAL CONVENTION & NATIONAL SYMPOSIUM
THRISSUR

28 - 30 November, 2012

DR K. S. ROY AWARD AND GOLD PLATED SILVER MEDAL FOR THE BEST PAPER IN HISTO-ENZYMOLOGY AND IMMUNO-HISTOCHEMISTRY

The Organizing Committee, XXVII Annual Convention of IAVA confers

DR K. S. ROY AWARD AND GOLD PLATED SILVER MEDAL FOR THE BEST PAPER IN HISTO-ENZYMOLOGY AND IMMUNO-HISTOCHEMISTRY

On Dr Devendra Pathak from GADVASU, Ludhiana

for the paper entitled Immuno Localization ... . of Estrous Cycle

authored by Devendra Pathak, Neelam Bansal, Kuldip Gupta & Ghuman SPS

and presented by Devendra Pathak

Dr. Ajay Prakash
Secretary

Dr. T. S. Chandrasekhara Rao
President

Place : Thrissur
Date : 30/11/2012

INDIAN ASSOCIATION OF VETERINARY ANATOMISTS

XXVIII ANNUAL CONVENTION & NATIONAL SYMPOSIUM

BIKANER

8 - 10 January, 2014

The Organizing Committee, XXVIII Annual Convention of IAVA confers

Dr. K. S. Roy Award for

BEST PAPER IN HISTO-ENZYMOLOGY AND IMMUNOHISTOCHEMISTRY

On Dr Devendra Pathak from Ludhiana

for the thesis entitled Immunolocalization of ------ of Buffalo

authored by Devendra Pathak, Neelam Bansal & Kuldip Gupta

and presented by Devendra Pathak

Place : Bikaner
Date : 10/01/2014

Dr. Ajay Prakash
Secretary

Dr. T. S. Chandrasekhara Rao
President

INDIAN ASSOCIATION OF VETERINARY ANATOMISTS

XXVIII ANNUAL CONVENTION & NATIONAL SYMPOSIUM

BIKANER

8 - 10 January, 2014

The Organizing Committee, XXVIII Annual Convention of IAVA confers

Dr. K. L. Suri Award & Medal for

BEST POSTER PRESENTATION

On Dr Devendra Pathak from Ludhiana

for the thesis entitled Immunolocalization - - - - - - - - - - of Buffalo

authored by Devendra Pathak, Neelam Bansal Kuldip Gupta & SPS Ghuman

and presented by Devendra Pathak

Place : Bikaner
Date : 10/01/2014

Dr. Ajay Prakash
Secretary

Dr. T. S. Chandrasekhara Rao
President

Annexure - III

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE IN RESPECT OF MAJOR RESEARCH PROJECT

1. Name of Principal Investigator : Dr. Devendra Pathak
2. Deptt. of University/College : Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana.
3. UGC approval No. and Date : F. No. 41-32/2012(SR) dated. 10 July 2012
4. Title of the Research Project : "Immounohistochemical Localization of estrogen and progesterone receptors in female genitalia of Buffalo"
5. Effective date of starting the project : 1 July 2012
6. (a)Period of Expenditure from : July 2012 to July 2015

b) Details of Expenditure

Sr. No.	Head	Sanctioned amount (Rs.)	Actual expenditure (Rs.)	Variations (Rs.)
A)	**Non-Recurring**			
	Books & Journals	NIL	NIL	NIL
	Equipment	NIL	NIL	NIL
B)	**Recurring** 1) Honorarium to Retd. Teacher @ Rs. 12000/-p.m.	NIL	NIL	NIL
	2)Wages/Project Fellow @ 14000/- p.m. initial 2 years and Rs.16000/- p.m. from the third year onwards.	NIL	NIL	NIL
	3) Any other Item	NIL	NIL	NIL
	4) Chemical/Glassware/Consumable	5,40,000/-	5,39,993/-	7/-
	5) Hiring Services	90,000/-	89,998/-	2/-
	6) Contingency/Other Charges	90,000/-	90,000/-	NIL
	7) Travel/Field work	22,500/-	14,893/-	7,607/-
	8) Overhead Charges @ Rs.10% approved recurring grant (except Travel & Field Work)	80,000/-	80,000/-	NIL
	Total (A+B)	**8,22,500/-**	**8,14,884/-**	**7,616/-**

Annexure – III(Contd.)

(c) Staff : No Staff appointed during the period July 2012 to July 2015

Date of Appointment : NA

S. No.	Expenditure Incurred From to	Amount Approved (Rs.)	Expenditure Incurred (Rs.)
1.	Honorarium to PI (Retired Teachers) Rs.12,000/- p.m.		nil
2.	Post-Doctoral Fellow Fellowship @ Rs. 12,000/- p.m.		nil
3.	Project Associate salary @ Rs.10,000/- p.m.		nil
4.	Project Fellow salary @ Rs.14,000/- p.m.		nil

1. It is certified that the appointment(s) have been made in accordance with the terms and conditions laid down by the Commission.

2. If as a result of check or audit objection, some irregularly is noticed at a later date, action will be taken to refund, adjust or regularize the objected amounts.

3. Payment @ revised rates shall be made with arrears on the availability of additional funds.

4. It is certified that the grant of Rs **8,22,500/-** (Rupees Eight Lakh Twenty two Thousand Five Hundred only) received from the University Grants Commission under the scheme of support for Major Research Project entitled **"Immunohistochemical localization of estrogen and progesterone receptors in female genitalia of Buffalo"** vide UGC letter No **F. No. 41-32/2012(SR) dated. July 10, 2012** from which an amount of Rs **8,14,884/-** has been utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission and an amount of Rs**7,616/-** has left unspent.

Signature of principal investigator

Head of the Department
Veterinary Anatomy
GADVASU, Ludhiana-141004

Dean
College of Veterinary Science

Director of Research
GADVASU, Ludhiana

Comptroller
GADVASU, Ludhiana

Deputy Controller(LA)
GADVASU, Ludhiana

DEPTT. OF VETY. ANATOMY GADVASU, LUDHIANA

Name/Title of the Scheme:- " Immunohistochemical localization of estrogen and progesterone receptors in female genitalia of Buffalo."

EXPENDITURE STATEMENT FOR THE YEAR 2015-16

Sr. No.	Head	Sanctioned amount (Rs.)	Actual expenditure (Rs.)	Variations (Rs.)
1.	**Contingency** Office Expenses (Recurring)	40,000/-	40,000/-	NIL
2.	**Material & Supply** Chemicals/Glasswares/Consumables	2,40,003/-	2,39,996/-	7/-
3.	**Other Charges** Hiring Services	40,019/-	40,017/-	2/-
4.	**Travel/Field**	10,094/-	2,487/-	7,607/-
	Total	**3,30,116/-**	**3,22,500/-**	**7,616/-**

Signature of principal investigator

Head of the Department
Veterinary Anatomy

Dean
College of Veterinary Science

Director of Research
GADVASU, Ludhiana

Comptroller
GADVASU, Ludhiana

Deputy Controller(I.A)
GADVASU, Ludhiana

Annexure-IV

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE INCURRED ON FIELD WORK

Name/Title of the Scheme:- "Immunohistochemical localization of estrogen and progesterone receptors in female genitalia of Buffalo."
Name of the Principal Investigator : Dr. Devendra Pathak

Name of Place visited	Duration of visit	Mode of Journey	Expenditure Incurred (Rs.)
Gajipur, Slaughter House and AIIMS New Delhi	27-01-2013 to 28-01-2013	By Train	1140/-
AIIMS New Delhi	19-5-2013 to 21-5-2013	By Train	2875/-
Gajipur, Slaughter House, New Delhi	3-7-2013 to 4-7-2013	By Bus	960/-
Bikaner, Rajasthan	7-01-2014 to 11-01-2014	By Train	3431/-
AIIMS New Delhi	22-01-2014 to 23-01-2014	By Train	4000/-
UGC, New Delhi	31-01-2014 to 01-02-2014	By Train	
AIIMS New Delhi	17-11-2014 to 19-11-2014	By Train	2487/-
TOTAL			**14,893/-**

Certified that the above expenditure is in accordance with the UGC norms for Major Research Projects

Signature of principal investigator

Head of the Department
Veterinary Anatomy

Dean
College of Veterinary Science

Director of Research
GADVASU, Ludhiana

Comptroller
GADVASU, Ludhiana

Deputy Controller(I.A)
GADVASU, Ludhiana

Annexure - V

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002
Utilization certificate

Certified that the grant of Rs.**8,22,500/-** (Rupees Eight Lakh Twenty Two Thousand Five Hundred only) received from the University Grants Commission under the scheme of support for Major Research Project entitled **" Immunohistochemical localization of estrogen and progesterone receptors in female genitalia of Buffalo."** vide UGC letter No **F. No. 41-32/2012(SR) dated. July 10, 2012** from which an amount of Rs **8,14,884/-**has been utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission and an amount of Rs **7,616/-**has left unspent.

Signature of principal investigator

Dept. of Vety. Anatomy, College of Veterinary Science, GADVASU, Ludhiana-141004

Head of the Department
Veterinary Anatomy

Professor Cum-Head
Department of Veterinary Anatomy
GADVASU, Ludhiana-141004

Dean
College of Veterinary Sciences
GADVASU, Ludhiana
College of Veterinary Science

Director of Research
GADVASU, Ludhiana

Guru Angad Dev Veterinary & Animal Sciences University
Ludhiana.

Comptroller
Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana.
Comptroller
GADVASU,Ludhiana

Deputy Controller (L A)
Guru Angad Dev Veterinary & Animal Sciences University Ludhiana
Deputy Controller(LA)
GADVASU, Ludhiana

Annexure-VI

PROFORMA FOR SUPPLYING THE INFORMATION IN RESPECT OF THE STAFF APPOINTED UNDER THE SCHEME OF MAJOR RESEARCH PROJECT

UGC FILE NO.: F. No. 41- 32/2012(SR) dated July 10, 2012

YEAR OF COMMENCEMENT: 2012

Name/Title of the Scheme:- "Immunohistochemical localization of estrogen and progesterone receptors in female genitalia of Buffalo."

1.	Name of the Principal Investigator	: Dr. Devendra Pathak
2.	Name of the University/College	: Guru Angad Dev Vety. & Animal Sciences University, Ludhiana
3.	Name of the Research Personnel appointed	: No Staff appointed during the period July 2012 to July 2015.

Sr. No.	Qualifications	Year	Marks	%age
1.	M.A./M.Sc./M. Tech./M. Sc Biotechnology			
2.	M. Phil			
3.	Ph.D.			
4.	Academic qualification			
5.	Date of joining	NA		
6.	Date of Birth of Research Personnel			
7.	Amount of HRA, if drawn			
8.	Number of Candidates applied for the post			

CERTIFICATE

This is to certify that all the rules and regulations of UGC Major Research Project outlined in the guidelines have been followed. Any lapse on the part of the University will liable to termination of said UGC project.

Signature of principal investigator

Assistant Scientist
Deptt. of Vety. Anatomy,
College of Veterinary Science,
GADVASU, Ludhiana-141004

Head of the Department
Veterinary Anatomy

Professor & Head
Department of Veterinary Anatomy
GADVASU, Ludhiana-141004

Dean,
College of Veterinary Science

Dean
College of Veterinary Sciences
GADVASU, Ludhiana

Director of Research
GADVASU, Ludhiana

Director of Research
Guru Angad Dev Veterinary
& Animal Sciences University
Ludhiana.

Comptroller
GADVASU,Ludhiana

Comptroller
Guru Angad Dev Veterinary
& Animal Sciences University,
Ludhiana

Deputy Controller(LA)
GADVASU, Ludhiana

Deputy Controller (L A)
Finance & Accounts Cell
Guru Angad Dev Vety. & Animal
Sciences University Ludhiana

Annexure-VII

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

MAJOR RESEARCH PROJECT COPY OF THE SPECIMEN OF HOUSE RENT FOR PROJECT FELLOW

Certified that Shri/Dr **N/A** is paying House rent of Rs. **N/A** and is eligible to draw House rent Allowances @ **N/A** as per University.

04/01/18

Signature of principal investigator

Certified that Shri/Dr. **N/A** is not staying independently and therefore is eligible to draw House rent @ of Rs. **N/A** p.m. minimum admissible to a Lecture as per University Rules.

04/01/18

Signature of principal investigator

Certified that Shri/Dr.**N/A** has been provided accommodation in the Hostel. But He/she could not be provided with single seated flat type accommodation as recommended by the Commission, hostel fee @ Rs. **N/A** per month w.e.f. **N/A** is being charged from him/her.

04/01/18

Signature of principal investigator

Dept. of Vety. Anatomy,
College of Veterinary Science,
GADVASU, Ludhiana-141004

Signature of principal investigator

Head of the Department
Veterinary Anatomy

Dean,
College of Veterinary Science

Director of Research
GADVASU, Ludhiana

Comptroller
GADVASU, Ludhiana

Deputy Controller(LA)
GADVASU, Ludhiana

Registered Post

From

The Comptroller,
Guru Angad Dev Veterinary and Animal Sciences University,
Ludhiana.

To

Under Secretary,
University Grants Commission,
Ministry of Human Resource Development,
Govt. of India,
Bahadur Shah Zafar Marg,
New Delhi-110002

Memo.No.CVU/B-1/18/-9556-59
Dated Ludhiana the: -24-1-18

Subject: - Audited Utilization Certificate along with the field details of the UGC project, "**Immounohistochemical Localization of estrogen and progressive receptors in female genitalia of Buffalo**".

F. No. 41-32/2012 (SR) dated 10-07-2012.
Name of Principal Investigator: Dr. Devendra Pathak.

With reference to your office letter no. F.No. 41-32/2012 (SR) dated 07-12-2017, please find enclosed herewith the Audited Utilization Certificated along with the the field details duly signed by the Deputy Controller (Local Audit), Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Statutory Audit Authority.

It is requested that acceptance of the same, may be conveyed to this University at the earliest.

Comptroller
GADVASU
Ludhiana

Encl: As above

CC:

1. Director of Research, GADVASU.
2. Head, Department of Veterinary Anatomy, GADVASU.
3. CAO (Budget), College of Veterinary Science, GADVASU.

3958
25/1/18

Annexure IX

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

ज्ञान-विज्ञान विमुक्तये

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. **Title of the Project:** "Immunohistochemical localization of estrogen and progesterone receptors in female genitalia of buffaloes"

2. **Name and address of the principal investigator**:
   Dr. Devendra Pathak, Assistant Scientist, Department of Veterinary Anatomy, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab

3. **Name and address of the institution**: Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab

4. **UGC Approval Letter No. and date:** F.No. 41-32/2012 (SR) dated July 10, 2012

5. **Date of implementation:** July, 1 2012

6. **Tenure of the project:** three years (July 1, 2012 to June 30, 2015)

7. **Total grant allocated:** Rs. 09,05,000

8. **Total grant received:** Rs. 08,22,500

9. **Final expenditure:** Rs. 8,14,884

10. **Title of the project :** "Immunohistochemical localization of estrogen and progesterone receptors in female genitalia of buffaloes"

11. **Objectives of the project :**

    1) To characterize the distribution of estrogen and progesterone receptors in ovaries of buffaloes during follicular and luteal phases of estrous cycle

    2) To characterize the distribution of estrogen and progesterone receptors in oviduct uterus and vagina of buffaloes during follicular and luteal phases of estrous cycle.

    3) To characterize the distribution of estrogen and progesterone receptors in ovary, oviduct, uterus and vagina of buffaloes during different seasons.

4) To study the blood hormone level of estrogen and progesterone with respect to localization of receptors during follicular and luteal phase and during different seasons.

12. Whether objectives were achieved : Yes

13. Achievements from the project:

Awards for Papers Presentations at national level

1. **Best Paper presentation Award and Medal 2012** for "Immuno localization of Estrogen receptor in oviduct of buffalo during follicular and luteal phase of estrous cycle" in Histoenzymology and Immunohistochemistry.

2. **Best Poster presentation Award and Medal 2014** for "Immunolocalization of progesterone receptor in oviduct of buffalo".

3. **Best Paper presentation Award 2014 for** "Immunolocalization of estrogen receptor alpha in uterus of buffalo" in Histoenzymology and Immunohistochemistry".

14. Summary of the findings:

Present research work was undertaken to characterize the distribution of estrogen and progesterone receptors in ovaries, oviduct, uterus and vagina of buffaloes during follicular and luteal phases of estrous cycle during different seasons.

ERα was localized in various cell types of buffalo ovary differentially in different stage of the reproductive cycle. Specific immunostaining was observed with anti-ERα antibody in the nuclei of follicular cells of primordial follicles, primary follicles, secondary and tertiary follicles. No reaction was observed in ovarian surface epithelium. ERα immunostained cells were observed at the prepubertal, follicular phase, luteal phase and pregnant ovary. In primordial follicles nuclear reaction was observed in one or two granulosa cells of the follicle. Similar to the primordial follicles the ERα was localized the granulosa cells. The connective tissue around these follicles both in the superficial and deep stroma showed the strong reaction for ERα antibody. In the growing follicle and secondary follicle the reaction was strong. While in the tertiary follicles weak reaction was observed in the granulosa cells and theca cells.ERα was weak or absent in the cells of corpora lutea. The progesterone receptors as revealed by immunohistochemistry were localized in the nuclei of different groups of ovarian cells. PR was localized in follicular cells of pre antral and antral follicles, stroma of ovary, endothelial cells of blood vessels. One or two granulosa cells of primordial and primary follicles were PR positive and immunoreactions was moderate while no staining was observed in oocytes. Moderate nuclear reaction was observed in the stromal cells of ovarian cortex. In the antral follicles both granulosa cells as well as theca cells were immunostained for PR. In the obliterative

atretic follicles the invading stromal cells were highly positive for PR. No staining was observed in negative controls.

Based on the studies on the oviduct, it was concluded that the estrogen receptor alpha was distributed in all the segments of oviduct (infundibulum, ampulla, Isthmus and utero-tubal junction). In all the segments estrogen receptor was localized in the lamina epithelialis, stromal cells in the propria submucosa, tunica muscularis and tunica serosa. The reaction were higher during the follicular phase as comapared to the luteal phase. Immunogold labeling with anti ERα antibody confirmed the findings of Immunohistochemical study at sub cellular level. The higher expression during the follicular phase was directly correlated with the level of estrogen hormone. Thus the present study was helpful in understanding the dynamics of ERα in different segments and in different compartments of buffalo oviduct in relation to the circulating hormonal levels during follicular and luteal phases of estrous cycle. The progesterone receptor was localized in lamina epithelialis, propria submucosa, tunica muscularis and tunica serosa. The maximum localization was observed in lamina epithelialis where both ciliated and secretory cell types were positive for progesterone receptors. Percentage of positive cells varied during the follicular and luteal phases of estrous cycle. The immune localization of ER and PR was maximum in the, oviduct during the winter and spring seasons as compared to the summer and rainy season. These results have contributed to a better understanding of the respective roles of ERs and PRs in the oviduct, of buffalo.

It was concluded from the studies on the uterus that the ERα was localized in luminal epithelium, glandular epithelium, stromal cells and myometrium and few cells in perimetrium. The immuno staining was observed in the nucleus of cells. In lamina epithelialis immunoreactivity was higher in follicular phase as compared with luteal phase of estrous cycle. The PR was localized in luminal epithelium, glandular epithelium, stromal cells and endothelial cells of blood vessels present in the propria sub mucosa and myometrium and few cells in perimetrium of buffalo uterus during the follicular and luteal phases of estrous cycle. Thus estrogen and progesterone play a major role in controlling the physiology of uterus by acting through their respective nuclear receptors.

The uterine cervix is a well-known target tissue for sex steroids as it contains specific protein receptors (ER and PR) for estrogen and progesterone. The present investigation aimed at determination of distribution of estrogen in cervix uteri of cyclic Indian buffalo during follicular and luteal phases of estrous cycle. The estrous cycle stage of 12 buffaloes was assessed by gross and histological appearance of ovaries. The tissue distribution of ER alpha was determined by immunohistochemical technique using one step polymer horseradish peroxidase staining system. Nuclear staining for ERα was observed in the epithelial cells of the surface epithelium, stromal cells and smooth muscle cells. In the cervix, ERα immuno reactivity was more intense in the epithelial, cervical

glands and smooth muscle cells during the follicular phase. These results indicated that the frequency and intensity of ERα immunoreactivity in the cervix of buffaloes varied according to the cervical cell types and the phases of the sexual cycle. No immunostaining was observed in any compartment of cervix uteri when the primary antibody was replaced by antibody diluent or buffer which served as negative control. The tissue distribution of PR was determined by immunohistochemical technique using one step polymer horseradish peroxidase staining system. Nuclear staining for PR was observed in the epithelial cells of the surface epithelium, stromal cells and smooth muscle cells. The lining epithelium of cervical glands showed intense positive nuclear reaction for progesterone receptor. These results indicated that the frequency and intensity of PR immuno reactivity in the cervix of buffaloes varied according to the cervical cell types and the phases of the sexual cycle. Immuno localization of Ki67 and PCNA was used to determine the relation of proliferation potential of estrogen hormone.

The results indicated that the status of cervix uteri using expression of proliferation marker PCNA and Ki67 was well correlated to the ERα expression in cervix uteri of buffalo. The ER and PR distribution in the vagina was significantly higher during follicular phase as compared to the luteal phase of estrous cycle.

The present study is useful for buffalo farmers to adopt a good breeding programme.

15. Contribution to the society:

Based on the project finding, famers are recommended to adopt estrous synchronization programme of buffaloes during the winter or spring seasons to get maximum fertility as the distribution of ER and PR in the reproductive tract is maximum and also the proliferative activity of the cells is maximum during this period. If the farmers are unable to synchronize during this period then a managemental programme to be adopted to decrease the summer stress. Based on the findings of present project it was possible to impart training to faculty members attending the CAFT Training Programme entitled "The cutting-edge technologies to enhance fertility in farm animals" from 4-24 Nov. 2016 held in Department of Animal Reproduction, Gynaecology and Obstetrics.

16. Whether any PH.D. Enrolled/produced out of the project: --NA—

17. No. Of publications out of the project :03+01+05=09

Annexure IX

Papers published/Submitted:

1. Pathak D, Bansal N, Singh O, Gupta K and Ghuman SPS. 2018. Immunohistochemical localization of Estrogen receptor alpha (ERα) in oviduct of buffalo during follicular and luteal phase of estrous cycle. *Tropical Animal Health and Production. TROP-D-18-01245 (In review).*
2. Pathak D, Bansal N, Singh O, Gupta K and Ghuman SPS. 2018. Immunolocalization of Progesterone Receptor (PR) in Oviduct of Indian Buffalo during Follicular and Luteal Phases of Estrous Cycle. *Journal of Animal Research (In review)*
3. Pathak D, Bansal N, Singh O, Gupta K and Ghuman SPS. 2018. Immuno Localization of Estrogen Receptor (ERα) and Progesterone Receptor (PR) in Uterus of Buffalo during Follicular and Luteal Phases of Estrous Cycle. *Turkish Journal of Veterinary and Animal Sciences. (In review).*

Training imparted in Centre for Advanced Faculty training:

- Devendra Pathak. 2016. ***Immunohistochemical techniques for the study of gonadal hormonal receptors*** **at CAFT Training Programme** "*The cutting-edge technologies to enhance fertility in farm animals"* from 4-24 Nov. 2016 held in Department of Animal Reproduction, Gynaecology and Obstetrics

Papers presented /Abstracts published

1. **Pathak D**, Bansal N, Gupta K and Ghuman SPS. 2012. **"Immuno localization of Estrogen receptor in oviduct of buffalo during follicular and luteal phase of estrous cycle"** at XXVIIth Annual Convention of Indian Association of Veterinary Anatomists and National Symposium at Department of Veterinary Anatomy and Histology, KAVASU, Thrissur from 28-30, November, 2012.

2. **Pathak D**, Neelam Bansal, Kuldip Gupta and SPS Ghuman. 2014. **"Immunolocalization of progesterone receptor in oviduct of buffalo"** *at XXVIIIth Annual Convention of Indian Association of Veterinary Anatomists and National Symposium* at Department of Veterinary Anatomy, Rajasthan University of Veterinary And Animal Sciences, Bikaner during 8-10, January, 2014.

3. **Pathak D**, Neelam Bansal and Kuldip Gupta. 2014. **"Immunolocalization of estrogen receptor alpha in uterus of buffalo"** at *XXVIIIth Annual Convention of Indian Association of Veterinary Anatomists and National Symposium* at Department of Veterinary Anatomy, Rajasthan University of Veterinary And Animal Sciences, Bikaner during 8-10, January, 2014.

4. **Pathak D**, Bansal N and Gupta K. 2014. "**Immunolocalization of estrogen receptor alpha and progesterone receptor in cervix uteri of Indian buffalo**" at *International conference on "Reproductive Health: Issues and strategies under changing climate scenario***"** and 24th Annual meet of ISSRF held from 6th – 8th February, 2014 at IVRI, Izatnagar.

5. **Pathak D** and Bansal N. 2016. "**Histomorphochemical features and progesterone receptor expression in atretic follicles in Indian buffalo"** Dr. S.S. Guraya Memorial Seminar of Advances in Animal Reproduction held at Dept. of Zoology, PAU Ludhiana 14100.

Signature of the
Principal investigator

Director of Research
(Seal)

Signature of the
Co-investigator

Signature of the
Head of the Department