RESEARCH ARTICLE

Expression of Estradiol-17β (ERβ) Receptor Protein in Whole Cortical Slices of Sheep Ovary

Supriya. B[1]*, A.V.N. Siva Kumar[2], K.V. Jamuna[3], Deepa P.[4], P. Jagapthi Ramayya[5]

Abstract

Estradiol 17β receptor (ERβ) protein expression in various cells of sheep ovaries was studied through immunohistochemistry. The ovaries recovered from eight adults apparently healthy sheep immediately after their slaughters were studied in the embryo biotechnology laboratory of Sri Venkateswara Veterinary University, Tirupati. The surface epithelial cells covering the ovary showed moderate immunoreactivity. The ovum of primordial follicles showed intense reaction and that of primary and secondary follicles showed moderate reaction. In early antral follicles, both ovum and granulosa cells showed moderate immunoreactivity. The theca cells were not immunoreactive. In antral follicles ovum and granulosa cells showed strong reactivity and theca interna cells showed mild to moderate reactivity. The cells of the corpus luteum showed moderate cytoplasmic immunoreactivity.

Key words: Cortical slice, Estradiol-17β receptor, Immunohistochemistry, Sheep ovary.

Ind J Vet Sci and Biotech (2024): 10.48165/ijvsbt.20.6.26

Introduction

Estradiol 17β is a key steroid hormone, proven to have a role in antrum formation, preovulatory follicle maturation (Emmen *et al*., 2005), expression of genes involved in ovarian differentiation, follicular rupture during ovulation of mammals, and in antrum formation of preantral follicles cultured *in vitro* (Tasaki *et al*., 2013). Since for the action of any hormone on the target cell and to have local action on any tissue, distribution, and expression of its receptor subtypes is necessary. Estrogen receptor beta belongs to the nuclear receptor superfamily (Mangelsdorf *et al*., 1995). Cardenas *et al*. (2001) identified the reading frame of ovine ERβ as a protein composed of 527 amino acids and ERβ expression in a few cells in the sheep ovary during the estrous cycle and early pregnancy. There is a dearth of information on the expression of the ERβ in granulosa cells and ova of different follicle sizes, *i.e.,* preantral, early antral, antral, and large antral follicles of sheep ovaries. Therefore, this study was aimed at the expression of estradiol-17β receptor protein in the cortical slices of sheep ovaries by immunohistochemistry.

Materials and Methods

Collection of Ovaries and Preparation of Cortical Slices

The ovaries were recovered from eight adult, apparently healthy, sheep immediately after their slaughter, and were transported to the embryo biotechnology laboratory of Sri Venkateswara Veterinary University, Tirupati (AP, India) within 1 h after slaughter in sterile, warm (37°C) phosphate-buffered saline. Then the cortical slices were separated using a sterile surgical blade and fixed in 10% neutral buffered formalin for 24h. The fixed slices were washed, dehydrated in ascending grades of alcohol, cleared in xylene, and embedded in paraffin. The sections of 5 µm thickness were obtained on clean, APES (Amino Propyl Ethoxy Sialine) coated glass slides with the help of Leica semi-automatic microtome (Leica RM2125RTS).

Immunohistochemistry

The sections were incubated overnight at 37°C. These tissue sections were subjected to the immunohistochemistry protocol

[1]Department of Veterinary Anatomy, College of Veterinary Science, Proddatur-516360, Sri Venkateswara Veterinary University, Andhra Pradesh, India

[2]Department of Veterinary Physiology, College of Veterinary Science, Tirupati-517502, Sri Venkateswara Veterinary University, Andhra Pradesh, India

[3]Department of Veterinary Anatomy and Histology, Veterinary College, Hebbal, Bengaluru-560024K,VAFSU, Karnataka, India

[4]Embryo Biotechnology Laboratory, Sri Venkateswara Veterinary University, Tirupati-517502, Andhra Pradesh, India

[5]College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati-517502, Andhra Pradesh, India

Corresponding Author: Supriya. B., Associate Professor, Department of Veterinary Anatomy, College of Veterinary Science, Proddatur-516360, SVVU, Andhra Pradesh, India. e-mail: supriyabotlagunta@gmail.com

How to cite this article: Supriya, B., Siva Kumar, A. V. N., Jamuna, K. V., Deepa, P., & Jagapthi Ramayya, P. (2024). Expression of Estradiol-17β (ERβ) Receptor Protein in Whole Cortical Slices of Sheep Ovary. Ind J Vet Sci and Biotech. 20(6), 138-141.

Source of support: Nil

Conflict of interest: None

Submitted: 23/07/2024 **Accepted** 09/09/2024 **Published** 10/11/2024

of Lynch *et al.* (1969). The immunohistochemical labelling was examined using a Micaps Pro series 1080 HDMI camera.

Follicles were classified according to the stage of development, considering the shape, and layers of the granulosa cells (GC) as primordial (oocyte surrounded by a flat pre-granulosa cell layer), primary (one layer of cuboidal granulosa cells), secondary (two or more layers of cuboidal cells, but with the formation of vesicles), early antral (three or more layers of cuboidal granulosa cells and the presence of antrum) and large antral follicles according to Da Silva-Buttkus *et al*. (2008).

Results and Discussion

In the present study, the intensity of reactivity was categorized as mild, moderate, and strong. In the paraffin sections of the ovary, ERβ immunoreactivity was observed both in follicles of all stages of development and in corpora lutea. In the sheep ovaries, ER β staining was observed in granulosa cells of all follicles. However, in large preovulatory follicles, more cells were positively stained than in the early antral ones.

Localization of ovine ERβ in granulosa cells and oocytes was consistent with the earlier reports of Cardenas *et al*. (2001) in sheep and of Enmark *et al*. (1997), Fitzpatrick *et al*. (1999) and Rosenfeld *et al*. (1999) in other animals. The ovarian surface epithelium showed moderate immunoreactivity in a few cells (Fig. 1). These results were in agreement with the results of Juengel *et al*. (2006) in sheep ovaries during prenatal and adult stages. The ERβ immunoreactivity was reported earlier in the surface epithelium of the ovary in humans (Brandenberger *et al*., 1998) and cynomolgus monkeys (Pelletier *et al*., 1999). ERβ protein was observed in stromal cells, particularly in areas containing numerous small follicles, which were in line with the findings of Juengel *et al*. (2006).

The ovum of the primordial follicles showed an intense reaction, whereas surrounding squamous pre-granulosa cells were negative (Fig. 1), whereas, Pelletier *et al*. (1999) stated that primordial follicles did not show any labelling for ERβ mRNA. The differences in the immunostaining of ERβ may be due to methodologic considerations, such as differences in antigen retrieval or the use of different fixatives and antibodies with varied sensitivity in different species.

The ovum of the primary follicle and secondary follicle showed moderate immunoreactivity. In the secondary follicle, only a few granulosa cells showed mild to moderate immunostaining. Oocytes revealed a lack of nuclear immunoreactivity, but their cytoplasm was moderately stained (Fig. 3). The oocyte of the primordial follicle, primary follicle, secondary follicle, and antral follicle showed ER β mRNA in the adult baboon ovary (Bocca *et al*., 2008).

In early antral follicles, both ovum and granulosa cells showed moderate immunoreactivity. The theca cells were not immunoreactive. The number of granulosa cells showing immunoreactivity also increased (Fig. 4; Fig. 5). The ERβ has been consistently detected in the membrana granulosa of follicles of different species including rats (Fitzpatrick *et al*., 1999; O'Brien *et al*., 2003), mice (Drummond *et al*.,1999), pig (Slomczynska and Wozniak, 2001), cattle (Rosenfeld *et al*., 1999), sheep (Cardenas *et al*., 2001), marmosets (Saunders *et al*., 2000) and humans (Enmark *et al*., 1997).

In antral follicles ovum and granulosa cells showed strong reactivity and theca interna cells showed mild to moderate reactivity. In antral follicles, ERβ appeared to be evenly distributed in granulosa cells throughout the follicle and in the current series of samples, no evidence for higher levels of expression of ERβ protein in mural granulosa cells were found compared to those within the cumulus. As the follicle grew in size, the intensity of reactivity increased in the ovum (Fig. 7, Fig. 9 & Fig. 10). In large antral follicles of bovine, positive staining was present in the corona radiata, cumulus oophorus, and mural granulosa cells, and slight staining in both theca interna and theca externa cells (Rosenfeld *et al.,* 1999).

In the present study, till the early antral stage, the follicles did not show immunoreactivity in the theca cells. In the large antral follicles, the intensity of immunostaining in the theca interna was moderate in line with Juengel *et al.* (2006), whereas theca externa did not show any immunostaining. The immunohistochemistry in the rat ovary demonstrated weak positive staining within theca cells (Saunders *et al*., 1997), but *in situ* hybridization in rat and human ovaries did not demonstrate specific expression of ERβ mRNA (Enmark *et al*., 1997). Contrary to this *in situ* hybridization for ERβ in cynomolgus monkeys showed strongly labelled theca interna cells (Pelltier *et al*., 1999). ERβ was not detected in the theca cells of the baboon ovary (Pepe *et al*., 2002). ERβ was detected in the theca interna of cycling pigs (Slomczynska and Wozniac, 2001) and low amounts of ERβ mRNA in theca cells have been found in rat and human ovaries (Saunders *et al*., 2000).

The cells of the corpus luteum showed moderate cytoplasmic immunoreactivity (Fig.15) in line with the findings of Juengel *et al.* (2006).The intensity of ERβ staining in the CL increased gradually as the pregnancy advanced 10 to 18 days in pigs as the conceptus-derived estrogen upregulates the expression of its receptors (Knapczyk *et al.,* 2008). The intensity of ERβ immunoreactivity in the granulosa cells of atretic follicles varied. Some cells showed moderate immunoreactivity and some cells completely lack staining (Fig.11). The interstitial cells showed moderate cytoplasmic immunoreaction. In the medulla, the endothelium of the blood vessels showed a positive reaction (Fig.12, Fig.13). The oestrogen regulates the transcription of Vascular endothelial growth factor (VEGF), a potent inducer of angiogenesis (Hyder *et al.,* 2000). Negative control sections incubated with rabbit serum showed that serum proteins caused very weak nonspecific staining (Fig. 2, Fig. 6, Fig. 8, Fig.14, & Fig.16) and there were no signs of nuclear reaction. In human corpora luteum (CL), estrogenic activity is mediated by ERβ with both protein and m-RNA localized to luteal cells, perivascular cells, and fibroblasts within the CL (Hosokawa *et al*., 2001).

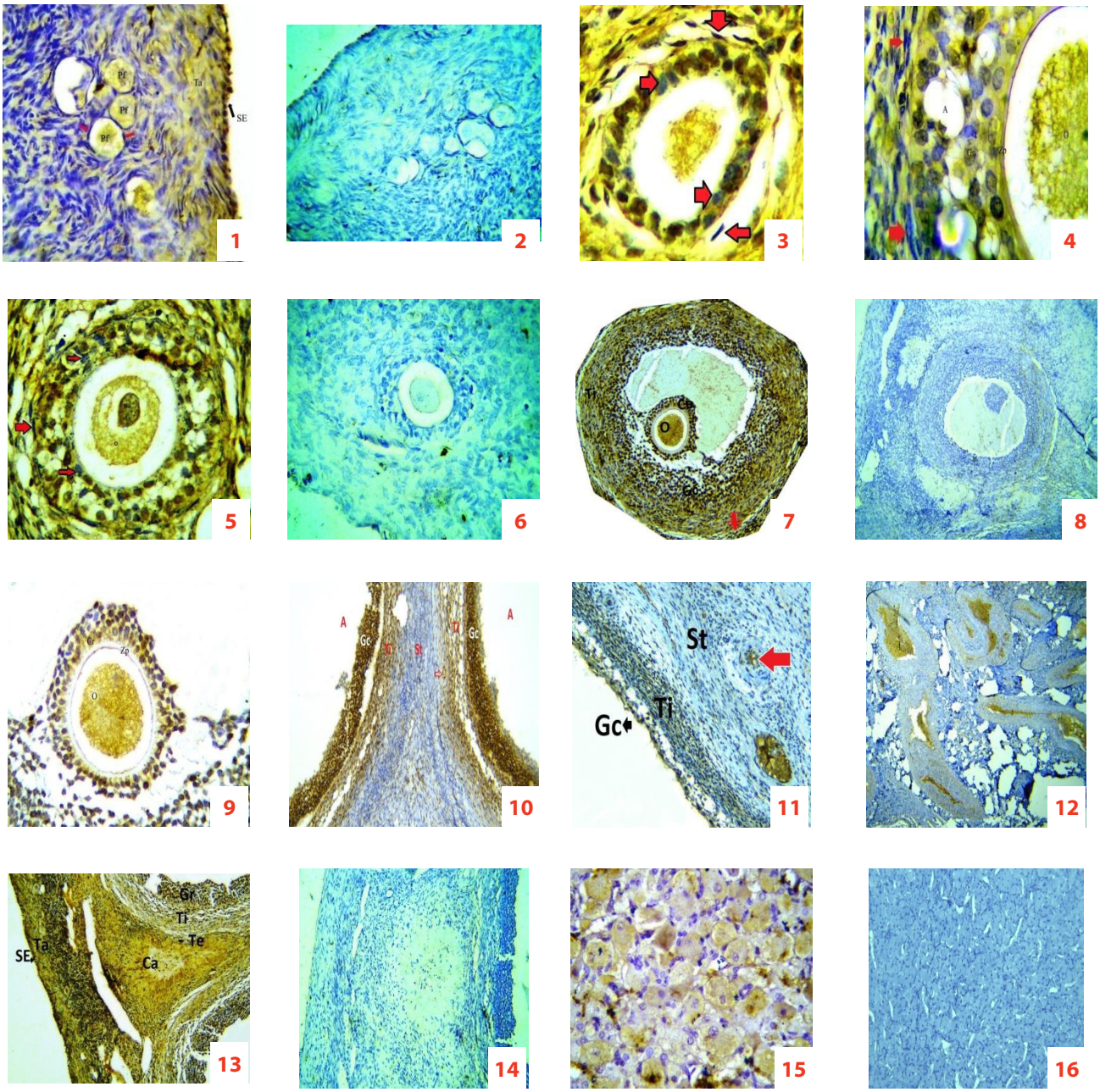

Fig.1: Photomicrographs showing Immunoreactivity of surface epithelium and ova of primordial follicles. The pregranulosa cells did not show immunoreactivity (red arrow). **Fig. 2**: Negative control for surface epithelium and primordial follicles of sheep ovary. **Fig. 3**: The primary follicle shows moderate immunoreactivity in the oocyte. The granulose cells showed mild to moderate reactivity only in a few cells. Some granulosa cells were negative (arrow). **Fig. 4**: Immunoreactivity of the secondary follicle. Oocyte and almost all granulose cells were moderately positive. Only a few granulose and theca cells were negative (arrow). **Fig. 5:** Strong nuclear immunostaining and moderate cytoplasmic staining in the ovum of the early antral follicle. **Fig. 6**: Negative control for the secondary follicle. **Fig. 7:** Immunoreactivity of the antral follicle. The ovum and granulosa cells showed strong reactivity and theca interna cells showed moderate reactivity. Theca externa was negative (red arrow). **Fig. 8:** Negative control for the antral follicle. **Fig. 9:** Strong immunoreactivity in the oocyte, corona radiate, and cumulus cells of large antral follicles. **Fig. 10**: The strong immunostaining of the entire mural granulose of large antral follicles. The theca internal cells were moderately positive. The endothelium of tunica interna was positive (arrow). **Fig. 11:** Immunoreactivity of the atretic antral follicle. The endothelium of the stromal vessel shows positive immunoreactivity (arrow). **Fig. 12**: The immunoreactivity of the medulla. **Fig. 13:** Immunoreactivity of carpus albicans and wall of large antral follicles. **Fig. 14:** Negative control for stroma, corpus albicans, and wall of the large antral follicle. **Fig. 15:** The cytoplasmic reactivity of luteal cells of corpus luteum**. Fig. 16:** Negative control for corpus luteum.

Acknowledgment

This work was supported by a research grant from the Science and Engineering Research Board (DST No: EMR/2017/000851) to A.V.N. Siva Kumar.

References

Bocca, S.M., Billiar, R.B., Albrecht, E.D., & Pepe, G.J. (2008). Oocytes of baboon foetalprimordial ovarian follicles express estrogen receptor β mRNA. *Endocrine*, *33*(3), 254-260.

Brandenberger, A.W., Tee, M.K., & Jaffe, R.B. (1998). Estrogen receptor alpha (ER-α) and beta (ER-β) mRNAs in normal ovary, ovarian serous cyst adenocarcinoma and ovarian cancer cell lines: Down-regulation of ER-β in neoplastic tissues. *The Journal of Clinical Endocrinology and Metabolism, 83*(3), 1025-1028.

Cardenas, H., Burke, K.A., Bigsby, R.M., Pope, W.F., & Nephew, K.P. (2001). Estrogen receptor β in the sheep ovary during the estrous cycle and early pregnancy. *Biology of Reproduction, 65*(1), 128-134.

Da Silva-Buttkus, P., Jayasooriya, G.S., Mora, J.M., Mobberley, M., Ryder, T.A., Baithun, M., Stark, J., Franks, S., & Hardy, K. (2008). Effect of cell shape and packing density on granulosa cell proliferation and formation of multiple layers during early follicle development in the ovary. *Journal of Cell Science*, *121,* 3890-3900.

Drummond, A.E., Baillie, A.J., & Findlay, J.K. (1999). Ovarian estrogen receptor α and β mRNA expression: Impact of development and estrogen. *Molecular and Cellular Endocrinology*, *149*(1-2), 153-161.

Emmen, J.M., Couse, J.F., Elmore, S.A., Yates, M.M., Kissling, G.E., & Korach, K.S. (2005). *In vitro* growth and ovulation of follicles from ovaries of estrogen receptor (ER) α and ERβ null mice indicate a role for ERβ in follicular maturation. *Endocrinology*, *146*(6), 2817-2826.

Enmark, E., Pelto-Huikko, M., Grandien, K., Lagercrantz, S., Lagercrantz, J., Fried, G., Nordenskjold, M., & Gustafsson, J.A.(1997). Human estrogen receptor beta gene structure, chromosomal localization, and expression pattern. *Journal of Clinical Endocrinology and Metabolism*, *82,* 4258-4265.

Fitzpatrick, S.L., Funkhouser, J.M., Sindoni, D.M., Stevis, P.E., Deecher, D.C., Bapat, A.R., Merchenthaler, I., & Frail, D.E. (1999). Expression of estrogen receptor-β protein in rodent ovary. *Endocrinology*, *140*(6), 2581-2591.

Hosokawa, K., Ottander, U., Wahlberg, P., Ny, T., Cajander, S., & Olofsson, I.J. (2001). Dominant expression and distribution of oestrogen receptor β over oestrogen receptor α in the human corpus luteum. *Molecular Human Reproduction*, *7*(2), 137-145.

Hyder, S.M., Zafar Nawaz, Constance Chiappetta, & George, M.S. (2000). Identification of functional estrogen response elements in the gene coding for the potent angiogenic factor vascular endothelial growth factor. *Cancer Research, 60*(2), 3183-3190.

Juengel, J.L., Heath, D.A., Quirke, L.D., & Mcnatty, K.P. (2006). Oestrogen receptor α and β, androgen receptor and progesterone receptor mRNA and protein localization within the developing ovary and in small growing follicles of sheep. *Reproduction*, *131*(1), 81-92.

Knapczyk, K., Duda, M., Durlej, M., Galas, J., Koziorowski, M., & Slomczynska, M. (2008). Expression of estrogen receptor β (ERβ) in the ovarian follicles and corpora lutea of pregnant swine. *Domestic Animal Endocrinology, 35,* 170-179.

Lynch, M.J., Raphael, S.S., Mellor, L.D., Spare, P.D., & Inwood, M.J.H. (1969). *Medical Laboratory Technology And Clinical Pathology*, 2nd edn. W.B. Saunders Company, London

O'Brien, M.J., Pendola, J.K., & Eppig, J.J. (2003). A revised protocol for *in vitro* development of mouse oocytes from primordial follicles dramatically improves their developmental competence. *Biology of Reproduction, 68*(5), 1682-1686.

Mangelsdorf, D.J., Thummel, C., Beato, M., Herrlich, P., Schütz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., & Evans, R.M., (1995). The nuclear receptor superfamily: The second decade. *Cell*, *83*(6), 835.

Pelletier, G., Luu-The, V., Charbonneau, A., & Labrie, F. (1999). Cellular localization of estrogen receptor beta messenger ribonucleic acid in cynomolgus monkey reproductive organs. *Biology of Reproduction, 61*(5), 1249-1255.

Pepe, G.J., Billiar, R.B., Leavitt, M.G., Zachos, N.C., Gustafsson, J.Å., & Albrecht, E.D. (2002). Expression of estrogen receptors α and β in the baboon fetal ovary. *Biology of Reproduction*., *66*(4), 1054-1060

Rosenfeld, C.S., Yuan, X., Manikkam, M., Calder, M.D., Garverick, H.A., & Lubahn, D.B. (1999). Cloning, sequencing, and localization of bovine estrogen receptor-β within the ovarian follicle. *Biology of Reproduction, 60*(3), 691-697.

Saunders, P.T.K., Maguire, S.M., Gaughan, J., & Millar, M.R. (1997). Expression of oestrogen receptor beta (ER) in multiple rat tissues visualized by immunohistochemistry. *Journal of Endocrinology*, *154,* R13-R16.

Saunders, P.T., Millar, M.R., Williams, K., Macpherson, S., Harkiss, D., Anderson, R.A., Orr, B., Groome, N.P., Scobie, G., & Fraser, H.M. (2000). Differential expression of estrogen receptor-α and-β and androgen receptor in the ovaries of marmosets and humans. *Biology of Reproduction, 63*(4), 1098-1105.

Słomczynska, M., & Wozniak, J. (2001). Differential distribution of estrogen receptor-β and estrogen receptor-α in the porcine ovary. *Experimental and Clinical Endocrinology & Diabetes*, *109*(4), 238-244.

Tasaki, H., Iwata, H., Sato, D., Monji, Y., & Kuwayama, T. (2013). Estradiol has a majorrole in antrum formation of porcine preantral follicles cultured *in vitro*. *Theriogenology, 79*(5), 809-814.