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RESEARCH ARTICLE

Immunohistochemical Detection of Estrogen and Progesteron Receptors in the Bovine Uterus and Their Relation to Serum Sex Steroid Hormone Levels During the Follicular and Luteal Phase

Berna GUNEY SARUHAN * Hakan SAGSOZ * M. Aydın KETANI *
M. Erdem AKBALIK * Nihat OZYURTLU **

* Department of Histology and Embrology, Faculty of Veterinary Medicine, University of Dicle, Diyarbakır - TURKEY
** Deparment of Obstetrics and Gynecology, Faculty of Veterinary Medicine, University of Dicle, Diyarbakır - TURKEY

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Summary

In this study, we tried to show the expression patterns of the steroid receptors in the bovine endometrium during the follicular and luteal phase. Samples of both uterus and blood were obtained from 30 adult, healty bovine at the moment of slaughter at a local slaughterhouse. Immunohistochemistry was performed by using rabbit polyclonal antibodies against the estrogen receptor (ER) and mouse monoclonal antibodies against the progesterone receptor (PR). In general, most of the uterine cells were stained positive but with a different intensity. During follicular phase, both ER and PR were obviously strong in the epithelia and the myometrium. For the glandular epithelium (GE), all GE cells were stained positive for ER and PR. When we compared both receptors during lutheal phase, a stronger intensity was observed in all compartments for PR, especially in the myometrium and surface-glandular epithelium. To summarize, the results from this study showed that both ER and PR might be regulated by the same mechanisms in some compartments and at specific stages of the oestrous cycle, and that each compartment of the uterus had a different expression of ER and PR which could accord with their different roles in reproductive physiology.

Keywords: *Immunohistochemistry, Estrogen receptor, Progesterone receptor, Bovine uterus*

İnek Uterusunda Folliküler ve Luteal Fazda Östrojen ve Progesteron Reseptörlerinin İmmunohistokimyasal Olarak Belirlenmesi ve Serum Steroidleri ile İlişkisi

Özet

Bu çalışma, foliküler ve luteal faz süresince inek endometriumunda steroid reseptörlerinin varlığını göstermek için planlandı. Kesimhaneye getirilen 30 adet erişkin ve sağlıklı inekten hem kan hemde uterus örnekleri alındı. İmmunohistokimyasal boyamada, östrojen reseptörü için tavşan poliklonal antikoru, progesteron reseptörü için ise fare monoklonal antikoru kullanıldı. Genel olarak, uterusun bütün bölümlerindeki hücrelerinde pozitif boyanma görüldü ancak yoğunlukları farklıydı. Foliküler faz süresince, epitel ve miyometriumda hem ER hemde PR için güçlü bir boyanma vardı. Bez epitel hücrelerinde hem ER hemde PR pozitif boyanmıştı. Luteal faz süresince her iki reseptör karşılaştırıldığında, uterusun bütün bölümlerinde özellikle miyometrium ile bez ve yüzey epitelinde PR için yoğun bir boyanma görüldü. Özetle, bu çalışmanın sonuçları gösterdi ki, hem ER hemde PR, östrus siklusunun bazı dönemlerinde benzer mekanizmalar tarafından düzenlenebilir ve uterusun her bir bölümünde ER ve PR nin farklı oranlarda bulunması, onların üreme fizyolojisindeki farklı rolleri ile ilişkilidir.

Anahtar sözcükler: *Immunohistokimya, Östrojen reseptör, Progesteron reseptör, İnek uterusu*

INTRODUCTION

Steroid hormones are important regulators of reproductive physiology in the female animals. Both oestradiol and progesterone mediate dramatic changes in bovine reproductive tissues during the oestrous cycle and early pregnancy. The actions of these steroid hormones are mediated through receptors located in the nuclei of cells [1]. Steroid hormones bind to receptors, and these ligand-receptor complexes serve as transcription

İletişim (Correspondence)
☎ +90 412 2488020/8627
✉ bernasaruhan@hotmail.com

factors that interact with DNA directly to regulate gene expression [2]. Ruminants, i.e., sheep, cattle, and goats, are spontaneous ovulators that undergo uterine-dependent estrus cycles until the establishment of pregnancy. Regular oestrus cycles, as well as establishment and maintenance of pregnancy, require integration of both endocrine and paracrine signals from the ovary, conceptus, and uterus itself [3]. Some biochemical investigations carried out on cow and sheep endometrium and myometrium during the oestrous cycle and pregnancy have demonstrated that the number of oestrogen (ER) and progesterone receptors (PR) varies in response to changes in blood concentration of endogenous sexual steroid hormones. In particular, it was shown that oestradiol stimulated a marked increase, while progesterone stimulated a decrease in the number of both receptor types [4,5].

The roles of ER in uterine function are well established. They include the induction of endometrial proliferation, in prepration for the onset of glandular secretion during the progesterone- dominated secretory phase, and modulation of luteolytic mechanisms through prostaglandin secretion at the end of the luteal phase of the non-pregnant cycle in animals with a uterine luteolysin [6].

Progesterone, synthesized and secreted by the corpus luteum, is one of the major regulators of the reproductive cycle in mammals. It exerts its effects on the growth and differentiation of ovarian structures and renders the endometrium receptive to the implantation of the embryo. Progesterone acts on its target tissues after binding to a specific intracellular PR [7,8].

In ruminants, the content of ER and PR in the uterus are greater at oestrus and less during the luteal phase in bovine [9-11] and sheep [3,12-14]. Estradiol has a stimulatory effect on the expression of ER and PR, whereas progesterone downregulates both receptors [13,15]. Immunohistochemical studies demonstrated that the distribution of receptors in the different uterine compartments varies in a cyclic manner during the bovine oestrous cycle in relation to plasma steroid hormone concentrations. However, cell types can display different sensitivities to oestrogens and progesterone, being the overall response of the uterus to steroid stimulation of the product of the combined responses of the various cell types [12].

The aim of the present study was to evaluate the distribution of ER and PR in tissue samples from bovine uterus during the oestrous cycle by immunohistochemistry. Finally, possible cyclic alterations to receptor localization in different cell types, and their correlation with serum progesterone and oestrogen level were determined.

MATERIAL and METHODS

Samples of both uterus and blood were obtained from 30 adult, healty bovine at the moment of slaughter at a local slaughterhouse. Serum samples collected immediately were stored at -20°C until assayed for determination of the serum concentrations of the sex steroids by using an ELISA. The concentrations of oestradiol (DRG EIA-2693) and progesterone (DRG EIA-1561) were measured. The corresponding serum oestrogen and progesterone levels of the animals were classified into two groups as follicular (n:10) and luteal (n:10) phase *(Fig 1)*. The uterus samples were collected at the moment of slaughter and fixed for 24 h in %10 neutral formaline.

Immunohistochemistry procedure

An indirect method (avidin-biotin-peroxidase) of immunohistochemical staining was used to evaluate the oestrogen and progesteron receptors. All tissue samples were embedded in paraffin wax, and sections of 5 µ were cut, mounted on 3-aminopropyl-triethoxysilane-coated (Sigma, St Louis, MO) slides and dried overnight at 37°C. Paraffin wax was removed with xylene; the sections were rehydratated and pre-treated in an Antigen Retrieval Citra Solution (0.01M, pH 6.0). The pre-treatment consisted of heating the slides for 30 min at 95°C. After cooling for 20 min at 40°C and rinsing in PBS, the slides were incubated for 30 min with 250 ml of a 3% (v/v) H_2O_2 solution to block endogenous peroxidase activity. Slides were rinsed in PBS and incubated with blocking reagent for 30 min at 25°C to prevent non-specific reactions. All incubations were carried out in a humidified environment. Immunohistochemical detection of oestrogen (Rabbit Polyclonal Antibody Ab-17, thermo scientific, cat. RB-1521-P) and progesterone receptors (Mouse Monoclonal Antibody Ab-2, thermo scientific, cat. MS-192-P) was performed. Each tissue section was incubated for 1 h at 25°C with a 1:200 dilution of the concentrated primary antibody (oestrogen and progesteron receptors) in antibodies diluent. After rinsing in PBS, the sections were incubated for 30 min at 25°C with a ready-to-use secondary antibodies (ER Labvision anti-rabbit ultravision large volume detection system TR-125-HL). Finally, after rinsing in PBS, 20 ml DAB chromogen substrate for ER and 20 ml

AEC chromogen substrate for PR was applied to the sections for 10 min. Gill haematoxylin for ER was applied for 1 min as a nuclear counterstain. Positive and negative controls were included in each staining procedure.

The stained slides were examined by using a research microscope and photographed with a digital camera attached to the microscope (Nikon Eclipsse-400, Colpix-4500).

Evaluation of the results

The expression of ER and PR in the uterine tissue was examined microscopically at x 200 magnification. The results of immunohistochemical staining were evaluated by semiquantitative methods. For the uterus, each of the uterine compartments, which consisted of the surface epithelium (SE), the connective tissue stroma (STR), the glandular epithelium (GE) and the myometrium (M) were analysed separately, and the results were shown according to the range of positive staining intensity. In each compartment, the staining intensity was evaluated at three different levels: +/weak intensity, ++/moderate intensity and +++/strong intensity. In some compartments, when the staining intensity was not uniform, the result was shown as a range: +/++ = weak to moderate and ++/+++ = moderate to strong intensity.

RESULTS

Serum hormone levels

The serum levels of oestrogen and progesterone changed during the oestrous cycle. The oestrogen level was high at follicular phase, whereas the progesterone level was high during luteal phase *(Fig 1)*.

Immunohistochemistry of ER in the uterus

In the uterus, positive staining for ER was observed as a reddish brown nuclear staining. No specific staining was found in the negative controls. Light-brown staining in the cytoplasm was consistently observed in the glandular epithelium and the surface epithelium at follicular and luteal phase. The intensity of specific nuclear staining varied not only between the follicular and luteal phase but also between the different uterine compartments. The endothelial and smooth muscle cells of the vessels in the endometrium and in the myometrium were not stained for ER. No staining was observed in the mesothelium. The intensity of positively stained nuclei of epithelial (surface and glandular), stromal and myometrial cells is presented for follicular and luteal phase of the oestrous cycle *(Table 1)*.

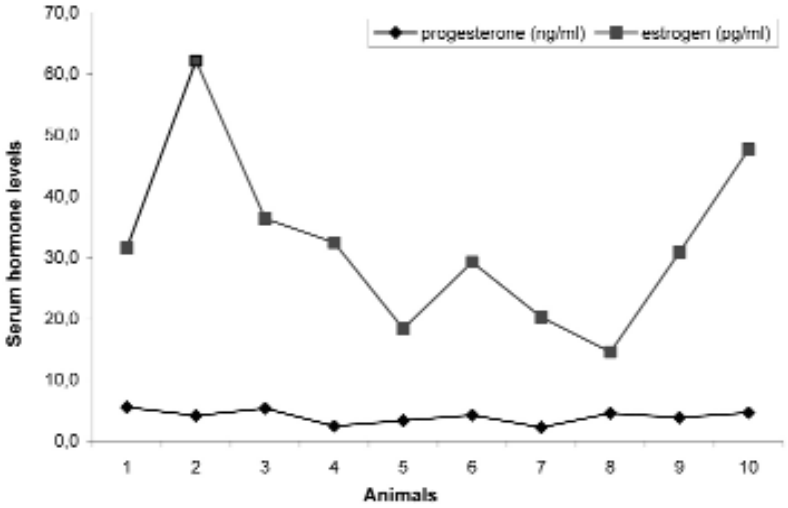

Fig 1. Serum levels of estradiol and progesterone (mean±SD) in bovine

Şekil 1. İneklerde serum da östradiol ve progesteron düzeyleri (mean±SD)

Table 1. *Immunohistochemical staining of oestrogen receptor (ER) presented as manual scoring (intensity) in different uterine tissue compartments at follicular and luteal phase*

Tablo 1. *Foliküler ve luteal fazda uterusun farklı bölümlerinde östrojen reseptörlerinin immunohistokimyasal boyanma skorları*

Stages	Surface epithelium	Endometrial glands		Stroma	Myometrium
		Superficial	Basal		
Follicular	++/+++	+++	++	++/+++	+++
Luteal	+/++	+/++	++	++	+/++

Staining intensity: + = weak, ++ = moderate, +++ = strong, +/++ = weak to moderate,
++/+++ = moderate to strong

For the follicular phase, the staining intensity of the positive cells was higher than that at luteal phase. In the surface epithelium (SE) at follicular phase, all epithelial cells were positively stained (moderate to strong intensity) for ER whereas no staining appeared

in the secretory cells *(Fig 2)*. For the connective tissue stroma of the subepithelial layer (functionale) of the endometrium, the results were observed to be moderate to strong immunostained ER positive cells. In the endometrial glands, the staining intensity always appeared stronger in the superficial glands than in the deep-laying glands. The strongest intensity of the glandular epithelium for both superficial and deep glands was found at follicular phase compared with the luteal phase. In the myometrium, the strongest intensity was observed during follicular phase *(Fig 3)*.

For the luteal phase, the staining intensity was lower than that at follicular phase. Positively stained nuclei of epithelial (surface and glandular), stromal and myometrial cells were determined at the luteal phase. Additionally, it was important to note that ER expression was more intense in the basal glands and stromal cells than in the superficial glands and stromal cells *(Fig 4)*.

Immunohistochemistry of PR in the uterus

Red nuclear staining demonstrating the presence of progesterone receptors was observed in 5 different cell groups, namely in epithelial cells of the surface epithelium, the endometrial glands (functional and basal), in endometrial stroma cells and in myometrial

Table 2. *Immunohistochemical staining of progesterone receptor (PR) presented as manual scoring (intensity) in different uterine tissue compartments at follicular and luteal phase*

Tablo 2. *Foliküler ve luteal fazda uterusun farklı bölümlerinde progesteron reseptörlerinin immunohistokimyasal boyanma skorları*

Stages	Surface epithelium	Endometrial glands		Stroma	Myometrium
		Superficial	Basal		
Follicular	+/++	++/+++	++/+++	+/++	+/++
Luteal	+/++	++/+++	++/+++	++	+++

Staining intensity: + = weak, ++ = moderate, +++ = strong, +/++ = weak to moderate,
++/+++ = moderate to strong

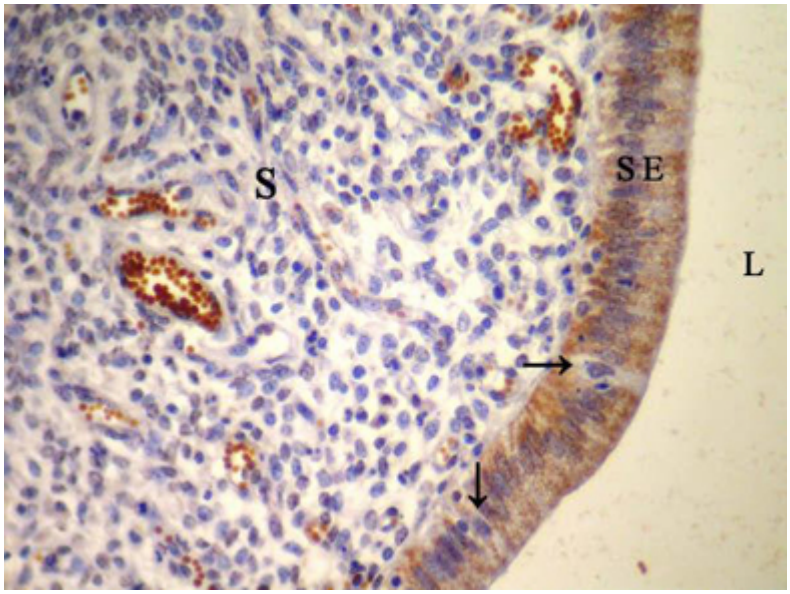

Fig 2. Immunohistochemical staining of ER in the surface epithelium at follicular phase. The nucleus of positive cells were stained brown color, and secretory cells were not stained, S: Stroma, SE: Surfece epithelium, L: Lumen, arrow: Secretory cell (x400)

Şekil 2. Foliküler fazda yüzey epitelinde ER için immunohistokimyasal boyanma. Pozitif boyanan hücrelerin kahverengi çekirdekleri, boyanmayan sekretor hücrelerin çekirdekleri, S: Stroma, SE: Yüzey epiteli, L: Lumen, ok: Sekretor hücre (x400)

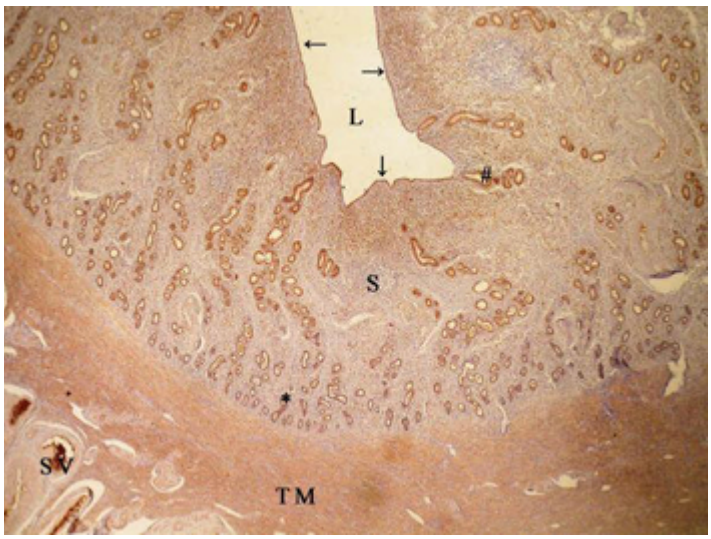

Fig 3. Immunohistochemical staining of ER in the surface epithelium, stroma, glands and tunica muscularis at follicular phase, L: Lumen, S: Stroma, TM: Tunica muscularis, SV: Stratum vasculosa, square: Superficial glands, star: Deep glands, arrow: Surface epithelium (x40)

Şekil 3. Foliküler fazda yüzey epiteli, stroma, bezler ve tunika muskulariste ER immunohistokimyasal boyanması, L: Lumen, S: Stroma, TM: Tunika muskularis, SV: Str. vaskuloza, Kare: Yüzeyel bezler, Yıldız: Derin bezler, ok: Yüzey epiteli (x40)

smooth muscle cells *(Table 2)*. Negative controls showed no staining. During the follicular and luteal phase, changes were observed in the staining for progesterone receptors. No positive staining for progesterone receptors was ever observed in the mesothelium, in endometrial blood vessel walls. Red staining in the cytoplasm of epithelial cells was observed occasionally but was considered to be nonspecific.

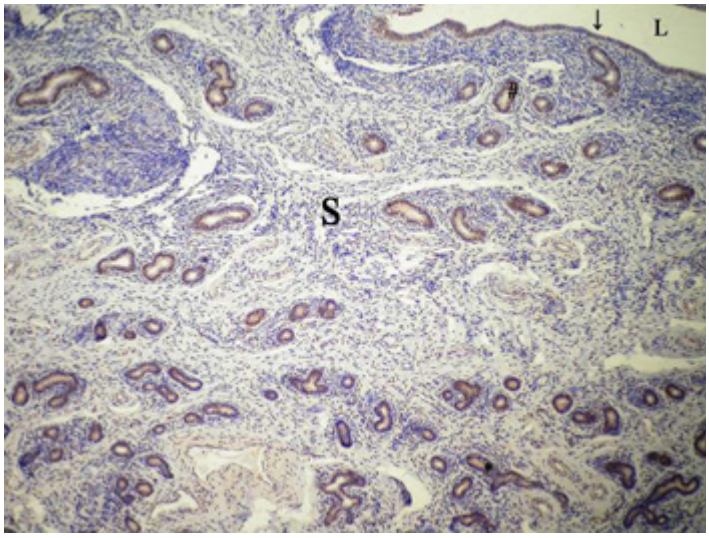

Fig 4. Immunohistochemical staining of ER in the surface epithelium, stroma, superficial and deep glands at luteal phase, L: Lumen, S: Stroma, square: Superficial glands, star: Deep glands, arrow: Surface epithelium (x100)

Şekil 4. Luteal fazda yüzey epiteli, stroma, bezlerde ER immunohistokimyasal boyanması, L: Lumen, S: Stroma, Kare: Yüzeyel bezler, Yıldız: Derin bezler, ok: Yüzey epiteli (x100)

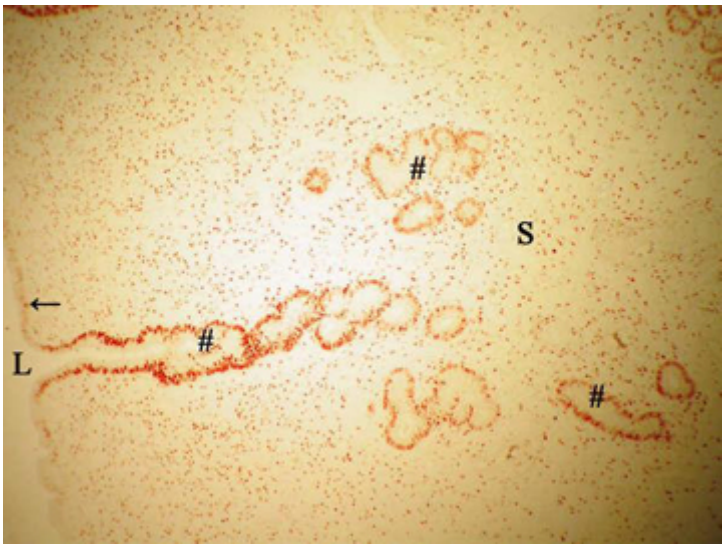

Fig 5. Immunohistochemical staining of PR in the surface epithelium, stroma and sureficial glands at follicular phase, L: Lumen, S: Stroma, square: Superficial glands, arrow: Surface epithelium (x100)

Şekil 5. Foliküler fazda yüzey epiteli, stroma, yüzeyel bezlerde PR immunohistokimyasal boyanması, L: Lumen, S: Stroma, Kare: Yüzeyel bezler, ok: Yüzey epiteli (x100)

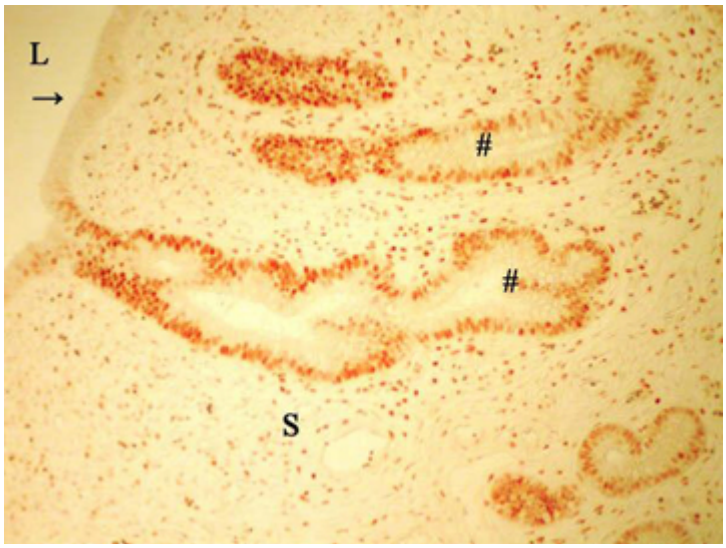

Fig 6a. Immunohistochemical staining of PR in the surface epithelium, stroma and superficial glands at luteal phase, L: Lumen, S: Stroma, square: Superficial glands, star: Deep glands, arrow: Surface epithelium (x200)

Şekil 6a. Luteal fazda yüzey epiteli, stroma, yüzeyel bezlerde PR immunohistokimyasal boyanması, L: Lumen, S: Stroma, Kare: Yüzeyel bezler, Yıldız: Derin bezler, ok: Yüzey epiteli (x200)

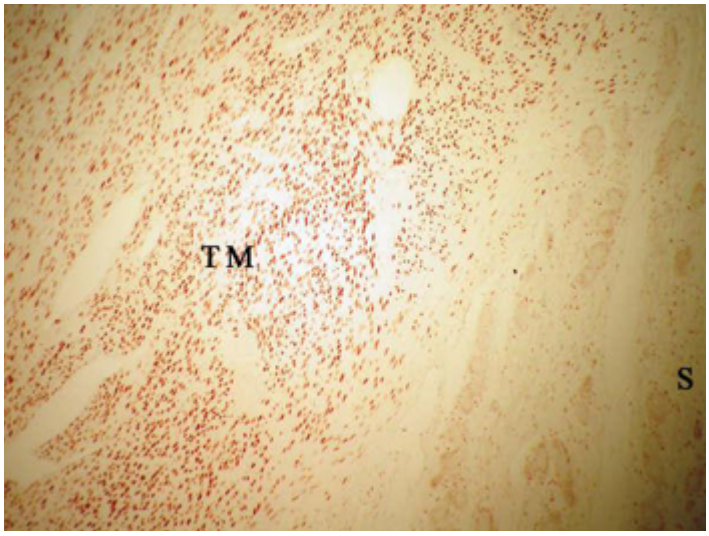

Fig 6b. Immunohistochemical staining of PR in the tunica muscularis at luteal phase, TM: Tunica muscularis, S: stroma (x200)

Şekil 6b. Luteal fazda tunika muskulariste PR immunohistokimyasal boyanması, TM: Tunika muskularis, S: stroma (x200)

For the follicular phase, the staining intensity in the surface epithelium was weak to moderate at PR-positive cells. The staining intensity in endometrial glands was moderate to strong and, was similar in the superficial and basal glands, which are simple, branched and coiled tubular glands. Almost all cells were PR-positive in the stroma. However, the intensities differed, being weak to moderate. The intensity of positive staining in the myometrium was weak to moderate *(Fig 5)*.

For the luteal phase, weak to modarete immunostaining was observed in the surface epithelium at PR-positive cells. The staining intensity in superficial and basal glands was moderate to strong in the endometrium. PR staining of the stromal cells were stained with moderate intensity. The intensity of positive staining in the myometrium was clearly strong *(Fig 6a, b)*.

DISCUSSION

Previous immunohistochemical research has demonstrated a species-specific and cell-specific expression of ER and PR in uterus tissues of different species [16-18]. In the present study, the expression of ER and PR in the bovine uterus was demonstrated in different cell groups. Furthermore, the possible correlation between plasma oestrogen and progesterone levels and receptor expression is discussed.

From the results of the present study, it was shown that both ER and PR might be up-regulated in the uterus by the high serum level of estrogen seen at follicular phase. This was consistent with many earlier studies that reported the positive effect of oestrogen on the expression of steroid receptors [10,11,19-21]. However, the level of estrogen may not be the only regulator to upregulate steroid receptors. This was confirmed by the ER and PR stainings in the epithelia which were still high during early luteal phase. Moreover, the stronger intensity in the myometrium for PR during early luteal phase, may be under the influence of progesterone, since progesterone treatment could result in myometrium hypertrophy [22,23]. This may be mediated by progesterone receptors in the myocytes when the level of progesterone is high. There are several studies showing that progesterone plays a major role in reproductive physiology associated with pregnancy via a progesterone receptor [24-26]. Therefore, withdrawal of PR in the SE should be observed during late dioestrus, when the bovine is not pregnant and is about to start a new oestrous cycle. Moreover, there was a study indicating that down-regulation of progesterone receptors in the uterine epithelium may be involved in the synthesis and release of prostaglandin F2 alpha (PGF2α), for the purpose of luteolysis [27].

In sheep, mice and rats, oestrogen influences expression of oestrogen and progesterone receptors in uterine stromal and epithelial cells [3,15,19,28,29]. In another study [10], presumably high levels of follicular oestrogen coincided with high levels of oestrogen and progesterone receptors. Maximal receptor levels can be attributed to oestrogen-up-regulated steroid receptor expression which has been well documented in other oestrogen target organs [30]. In the present study, for the stroma, most of the cells were stained positive, with a weak to strong intensity, during the oestrous cycle, which may indicate that steroid effects on the epithelial were mediated by stromal cells in a paracrine manner as described by other studies [11,21,26,31-35].

This was in agreement with many studies that reported that, during follicular phase, strong intensity was observed in the glandular epithelium for ER and PR [10,11,19-21]. In a study carried out on pig and monkey, it was reported that there was strong staining at the onset of luteal phase for both receptors, but a weak staining at the end of luteal [36,37]. We detected moderate to high reactivity in the endometrial glands as, reported by Kimmins et al.[10].

In conclusion, this study demonstrates the expression of ER and PR in the endometrium and myometrium of the bovine uterus during the follicular and luteal phase. Distribution and expression levels of steroid receptors are undoubtedly key to directing uterine cyclicity and the establishment of pregnancy in the bovine.

REFERENCES

1. Defranco DB: Navigating steroid hormone receptors through the nuclear compartment. *Mol Endocrin,* 16, 1449-1455, 2002.

2. Clarke CL: At the cutting edge. Cell-specific regulation of progesterone receptor in the female reproductive system. *Mol Cell Endocrin,* 70, 29-33, 1990.

3. Spencer TE, Bazer FW: Temporal and spatial alterations in uterine estrogen receptor and progesterone receptor gene expression during the estrous cycle and early pregnancy in the ewe. *Biol Reprod,* 53, 1527-1543, 1995.

4. Vesanen M, Isomaa V, Alanko M, Vikho R: Bovine uterine, cervical and ovarian androgen receptor concentrations. *Anim Reprod Sci,* 22, 268-275, 1991.

5. Klauke M, Hoffmann B: Progesterone and estrogen receptors in the myometrium of the cow during the estrous cycle and pregnancy and of the sheep at the time of parturition. *Anim Reprod Sci,* 18, 084-192, 1992.

6. Flint APF, Sheldrick EL, Fisher PA: Ligand-independent activation of steroid receptors. *Domest Anim Endocrin,* 23, 13-24, 2002.

7. Pinter JH, Deep C, Park-Sarge OK: Progesterone receptors: expression and regulation in the mammalian ovary. *Clin Obstet Gynecol,* 39, 424-435, 1996.

8. Dellmann HD, Eurell JA: Textbook of Veterinary Histology. Williams &Wilkins, London, 252-325, 1998.

9. Boos A, Meyer W, Schwarz R, Grunert E: Immunohistochemical assessment of oestrogen receptor and progesterone receptor distribution in biopsy samples of the bovine endometrium collected throughout the oestrous cycle. *Anim Reprod Sci,* 44,11-21, 1996.

10. Kimmins S, MacLarena LA: Oestrous cycle and pregnancy effects on the distribution of oestrogen and progesterone receptors in bovine endometrium. *Placenta*, 22, 742-748, 2001.

11. Robinson RS, Mann GE, Lamming GE, Wathes DC: Expression of oxytocin, oestrogen and progesterone receptors in uterine biopsy samples throughout the oestrous cycle and early pregnancy in cows. *Reprod,* 122, 965-979, 2001.

12. Cherny RA, Salamonsen LA, Findlay JK: Immunocytochemical localization of oestrogen receptors in the endometrium of the ewe. *Reprod Fert Develop,* 3, 321-331, 1991.

13. Wathes DC, Hamon M: Localisation of oestradiol, progesterone and oxytocin receptors in the uterus during the oestrous cycle and early pregnancy of the ewe. *J Endocrin,* 138, 479-491, 1993.

14. Spencer TE, Ott TL, Bazer FW: Expression of interferon regulatory factors one and two in the ovine endometrium: effects of pregnancy and ovine interferon tau. *Biol Reprod,* 58, 1154-1162, 1998.

15. Ing NH, Tornesi MB: Estradiol up-regulates estrogen receptor and progesterone receptor gene expression in specific ovine uterine cells. *Biol Reprod,* 56, 1205-1215, 1997.

16. Van Den Broeck W, Coryn M, Simoens P, Lauwers H: Cell-specific distribution of oestrogen receptor-alpha in the bovine ovary. *Reprod Dom Anim,* 37, 291-293, 2002.

17. Van Den Broeck W, D'Haeseleer M, Coryn M, Simoens P: Cell-specific distribution of progesterone receptors in the bovine ovary. *Reprod Domest Anim,* 37, 164-170, 2002.

18. Slomczynska M, Krok M, Pierscinski A: Localization of the progesterone receptor in the porcine ovary. *Acta Histochem,* 102, 183-191, 2000.

19. Wathes DC, Hamon M: Localization of oestradiol, progesterone and oxytocin receptors in the uterus during the oestrous cycle and early pregnancy of the ewe. *J Endocrinol,* 138, 479-492, 1993.

20. Dhaliwal GK, England GC, Noakes DE: Immunocytochemical localization of oestrogen and progesterone receptors in the uterus of the normal bitch during oestrus and metoestrus. *J Reprod Fertil (Suppl),* 51, 167-176, 1997.

21. Vermeirsch H, Simoens P, Hellemans A, Coryn M, Lauwers H: Immunohistochemical detection of progesterone receptors in the canine uterus and their relation to sex steroid hormone levels. *Theriogenology*, 53, 773-788, 2000.

22. De Bosscher H, Ducatelle R, Tshamala M, Coryn M: Changes in sex hormone receptors during administration of progesterone to prevent estrus in the bitch. *Theriogenology,* 58, 1209-1217, 2002.

23. Kamernitskii AV, Levina IS, Kulikova LE, Milovanov AP, Khalanskii AS, Altukhova VI, Smirnov AN, Pokrovskaia EV, Shevchenko VP: Morphological changes in the rat uterine tissues exposed to pregna-D'-pentaran derivatives of progestins and anti-progestins. *Bioorg Khim,* 28, 261-268, 2002.

24. Conneely OM, Mulac-Jericevic B, DeMayo F, Lydon JP, O'Malley BW: Reproductive functions of progesterone receptors. *Recent Prog Horm Res,* 57, 339-355, 2002.

25. Conneely OM, Mulac-Jericevic B, Lydon JP, De Mayo FJ: Reproductive functions of the progesterone receptor isoforms: lessons from knock-out mice. *Mol Cell Endocrinol,* 179, 97-103, 2001.

26. Spencer TE, Bazer FW: Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. *Front Biosci,* 7, 1879-1898, 2002.

27. Geisert RD, Morgan GL, Short EC Jr, Zavy MT: Endocrine events associated with endometrial function and conceptus development in cattle. *Reprod Fertil Dev,* 4, 301-305, 1992.

28. Kurita T, Lee K, Cooke PS, Taylor JA, Lubahn DB, Cunha GR: Paracrine regulation of epithelial progesterone receptor by estradiol in the mouse female reproductive tract. *Biol Reprod,* 62, 821-830, 2000.

29. Kurita T, Lee K, Cooke PS, Lydon JP, Cunha GR: Paracrine regulation of epithelial progesterone receptor and lactoferrin by progesterone in the mouse uterus. *Biol Reprod,* 62, 831-838, 2000.

30. Cunha GR, Young P: Role of stroma in oestrogen-induced epithelial proliferation. *Epith Cell Biol,* 1, 18-31, 1992.

31. Cooke PS, Buchanan DL, Young P, Setiawan T, Brody J, Korach KS, Taylor J, Lubahn DB, Cunha GR: Stromal estrogen receptors mediate mitogenic effects of estradiol on uterine epithelium. *Proc Natl Acad Sci USA,* 94, 6535-6540, 1997.

32. Buchanan DL, Kurita T, Taylor JA, Lubahn DB, Cunha GR, Cooke PS: Role of stromal and epithelial estrogen receptors in vaginal epithelial proliferation, stratification, and cornification. *Endocrin*, 139, 4345-4352, 1998.

33. Cooke PS, Buchanan DL, Lubahn DB, Cunha GR: Mechanism of estrogen action: Lessons from the estrogen receptor-alpha knockout mouse. *Biol Reprod,* 59, 470-475, 1998.

34. Buchanan DL, Setiawan T, Lubahn DB, Taylor JA, Kurita T, Cunha GR, Cooke PS: Tissue compartment-specific estrogen receptor-alpha participation in the mouse uterine epithelial secretory response. *Endocrin*, 140, 484-491, 1999.

35. Bigsby RM: Control of growth and differentiation of the endometrium: the role of tissue interactions. *Ann NY Acad Sci,* 955, 110-117, 2002.

36. Einspanier A, Bilefeld A, Kopp JH: Expression of the oxytocin receptor in relation to steroid receptors in the uterus of a primate model, the marmoset monkey. *Hum Reprod Update,* 4, 634-646, 1998.

37. Sukjumlong S, Srisuwatanasagul K, Adirekthaworn A, Sajjarengpong K: The expression of oestrogen and progesterone receptors in the gilt uterus at different stages of the oestrous cycle. *Thai J Vet Med,* 33, 3-30, 2003.