Reprod. Nutr. Dev. 40 (2000) 587–596

Original article

An immunohistochemical study on the regulation of estrogen receptor α by estradiol in the endometrium of the immature ewe

Ana MEIKLE[a,b,d]*, Alejandro BIELLI[c], Britt MASIRONI[d], Graciela PEDRANA[c], Hong WANG[d], Mats FORSBERG[b], Lena SAHLIN[d]

[a] Department of Biochemistry, Veterinary Faculty, Montevideo, Uruguay
[b] Department of Clinical Chemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden
[c] Department of Histology, Veterinary Faculty, Montevideo, Uruguay
[d] Department of Woman and Child Health, Division for Reproductive Endocrinology, Karolinska Institutet, Stockholm, Sweden

(Received 30 August 2000; accepted 30 November 2000)

Abstract — The effects of estradiol-17β (E2) on the expression of estrogen receptor α (ERα) in stromal and epithelial cells of endometrium in prepubertal lambs were investigated. Twenty three-month-old lambs were treated or not treated with one, two or three i.m. injections of E2 (1 $\mu g \cdot kg^{-1}$) in corn oil at intervals of 24 h. Lambs were slaughtered 12 or 24 h after the last injection. An immunohistochemical technique was used to visualize ERα immunostaining which was then analyzed quantitatively by a computer imaging analysis system. Seven endometrial compartments defined by cell type and location were analyzed separately. Positive staining of ERα was seen in the nuclei of stromal and epithelial cells. Glandular epithelium located next to the myometrium was stained more intensely than that next to the luminal epithelium and this phenomenon was maintained during treatment. Significantly less immunostaining was found in stromal cells 12 and 24 h after the first injection compared to the control group. A similar pattern was found in the glandular epithelium, although the decrease was more pronounced and the restoration of ERα was faster. This study shows that E2 treatment down regulates ERα in the endometrium temporarily in both stromal and epithelial cells, but the characteristics of this effect seems to be cell type specific.

estrogen / receptor / uterus / lamb / immunohistochemistry

Résumé — Étude immuno-cytologique de la régulation du récepteur aux œstrogènes ERα par l'œstradiol dans l'endomètre de la brebis impubère. Les effets de l'œstradiol-17β (E2) ont été étudiés sur l'expression du récepteur aux œstrogènes ERα dans les cellules du stroma et de l'épithélium de l'endomètre chez la brebis impubère. Vingt agnelles âgées de 3 mois ont reçu ou non une, deux ou

* Correspondence and reprints
E-mail: anamei@adinet.com.uy

trois administrations intramusculaires de E2 (1 μg·kg^{-1} en solution huileuse) à intervalle de 24 h. Elles ont été sacrifiées 12 ou 24 h après la dernière injection. ERα a été mis en évidence par immuno-cytologie et sa quantification a été faite à l'aide d'un système d'analyse d'images. Sept compartiments de l'endomètre, définis par leur type cellulaire et leur localisation, ont été analysés séparément. Un marquage positif a été constaté dans les noyaux des cellules du stroma et de l'épithélium. L'épithélium glandulaire situé prés du myomètre est marqué plus intensément que celui qui borde la lumière utérine et ce phénomène est encore observé à la suite des traitements. Un marquage significativement plus faible est constaté dans les cellules du stroma 12 et 24 h après la première injection par comparaison aux témoins. Une réponse identique est vue dans l'épithélium glandulaire bien que la diminution de marquage ait été plus prononcée et la restauration de ERα plus rapide. Cette étude montre que E2 régule négativement les récepteurs ERα de l'endomètre dans les cellules du stroma et de l'épithélium et cet effet semble être spécifique du type cellulaire.

œstradiol-17β / ERα / utérus / brebis impubère / immuno-cytologie

1. INTRODUCTION

Ovarian steroid hormones coordinate the function of the female reproductive tract acting primarily through intracellular proteins which are responsible for the recognition and transduction of the hormonal message. Estrogen receptors (ER) have been detected during mammalian embryonic and fetal stages, suggesting a role for estrogens in growth and differentiation at this stage of development [9, 10]. This has been confirmed by studies in ER knock-out mice which express abnormalities in the reproductive tract [14]. Two subtypes of ER have been described – ERα and ERβ – and ERα is predominant in the uterus [29]. The expression and regulation of ER has been studied in the reproductive tract during development in sheep and other species [8, 13, 15, 30]. Ruminant endometrium is especially interesting because it provides a model to study mechanisms by which a single mesodermally derived mucosal layer diverges into two different structural and functional areas: aglandular (caruncular) and glandular (intercaruncular) endometrium. Moreover, some studies have shown differences in sex steroid receptor expression and ovarian steroid hormone action along the reproductive tract between ruminants and other mammals [20, 26].

During development estrogens may have direct effects in cells that express ER but may also indirectly affect cells not expressing them [2]. Since the uterus is composed of different cell populations, it is important to localize the ER protein in the specific cell types. To our knowledge, no immunohistochemical studies of ERα expression and its regulation by estradiol-17β (E2) have been performed in ovine uterus before puberty. Few ERα positive nuclei in cervical cells of two prepubertal ewes were detected [31], which does not agree with a recent study where considerable amounts of ER were determined by binding assays (both ER subtypes measured) in both cervix and oviduct at this stage of development [18].

During the ontogeny of ERα in the genital tract of the mouse, ER was first observed in stromal cells, and although immunostaining of ERα in epithelial cells in all tissues increased with age, ERα immunostaining in stroma was almost constant with aging [30]. Moreover, DNA synthesis was stimulated in epithelial cells by E2 administration in neonatal mouse uteri, but ERα was localized in mesenchymal cells, and not in the epithelial cells [2]. It was suggested that stimulation of the epithelial cells could be caused by steroid hormones acting through a receptor mechanism in the underlying stroma, producing factors acting on

the epithelial cells [25], and this was recently confirmed [4, 6]. This type of crosstalk between stroma and epithelial cells was also reported for adult females; it was proposed that stromal cells contribute to epithelial reparation in human endometrium indirectly, presumably by their positive influence on growth factors and by providing cellular support to the newly resurfacing surface epithelial cells [3].

It was demonstrated in rodent uterus that the response – regarding protein synthesis and proliferation – to estrogen stimulation differs according to cell type [21, 22]. Moreover, the regulation of estrogen receptor expression by steroids is cell type specific in mature rodents [28], primates [3] and sheep [12]. Previous studies showed that E2 action on uterine ER and PR contents is biphasic in prepubertal lambs [17]. The early effect was dominated by receptor processing or loss of receptor protein, while the late replenishment phase seemed to be the result of an increase in the synthesis of new receptors. However, these data were obtained by binding assays and enzyme immunoassays in whole uterus [17], and could not address potential cellular changes that might have occurred. Since stromal-epithelial interactions play important roles in regulating the growth and differentiation of the endometrium during development and estrogens modulate these actions through their respective intracellular receptors, the effects of E2 on endometrial ERα expression in stromal and epithelial cells of prepubertal lambs were investigated in this study.

2. MATERIALS AND METHODS

2.1. Animals and hormone treatment

Twenty three-month-old, spring-born female Corriedale lambs (body weight, mean ± SEM: 17.4 ± 0.6 kg) were used. The lambs were kept under natural environmental conditions (extensive grazing) and were allowed to nurse freely during the experiment. Animals were randomly assigned to five groups (n = 4 each). Lambs were not treated (Group I, controls) or treated with one (Groups II and III), two (Group IV), or three (Group V) i.m. injections of E2 (1 $\mu g \cdot kg^{-1}$, Sigma, St.-Louis, MO, USA) in a corn oil vehicle at intervals of 24 h. The lambs were slaughtered at the beginning of the experiment (Group I), 12 h (Group II) or 24 h (Groups III, IV and V) after the last injection. At slaughter, the uteri were dissected promptly and upper, middle, and lower portions of the uterus were selected to obtain uniform samples of the whole uterine wall. The upper zone was defined as the portion of the uterus next to the oviduct, the lower zone as the portion of the uterus next to the cervix, and the middle zone as the area in between. Tissues were fixed immediately by immersion in Bouin's fixative solution for 24 h and thereafter stored in 70% ethanol until embedded in paraffin. Samples from one animal (Group II) were misprocessed and were not measured. Receptor determinations by ligand binding assays and enzyme immunoassays of this experiment have been published previously [17].

2.2. Immunohistochemistry

Paraffin sections (5 μm) from upper, middle and lower uterine zones in the five groups were prepared. An immunohistochemical technique (avidin-biotin-peroxidase) was used to visualize ERα immunostaining as described previously [29]. The monoclonal antibody used to detect ERα was ER C-311 raised against a protein corresponding to amino acids 495–595 at the carboxy terminus of the human ERα (cat# sc-787, Santa Cruz, California, US). After the tissue sections were dewaxed and rehydrated, an antigen retrieval procedure was performed. Sections were pretreated in a microwave oven at high power (700 W), in 0.01 M sodium citrate buffer (pH 6.0) for 10 min, and then allowed to cool for a further 20 min. After washing

in buffer (0.01 M PBS, pH 7.5) nonspecific endogenous peroxidase activity was blocked by treatment with 3% hydrogen peroxide (Merck) in methanol for 10 min at room temperature (RT). After a 10-min wash in buffer, sections were exposed to a 30-min non-immunoblock using diluted normal horse serum (Vectastain; Vector Laboratories, Burlingame, CA) in PBS in a humidified chamber at RT. The tissue sections were then incubated 60 min at RT with the primary antibody diluted 1:25 in PBS. Negative controls were obtained by replacing the primary antibody with mouse immunoglobulin G at equivalent concentration. After primary antibody binding, the sections were incubated for 60 min at RT with a biotinylated horse anti-mouse IgG (Vectastain, Vector) diluted in normal horse serum. Thereafter, the tissue sections were incubated for 60 min at RT with a horseradish peroxidase-avidin-biotin complex (Vectastain Elite; Vector). The site of the bound enzyme was visualized by the application of 3,3'-diaminobenzidine in H_2O_2 (DAB kit; Vector) a chromogen that produces a brown, insoluble precipitate when incubated with the enzyme. The sections were counterstained with hematoxylin and dehydrated before they were mounted with Pertex (Histolab, Gothenburg, Sweden).

2.3. Image analysis

After a general inspection of each slide, a quantitative image analysis was performed to estimate the expression of ERα in different cell types. A Leica microscope connected to a computer using Qwin software (Leica Imaging System Ltd.) was used to assess immunostaining on the digitized images of systematic randomly selected fields. ERα expression was measured in 7 uterine compartments defined by cell type and location. The glandular epithelium was arbitrarily divided in 3 portions: superficial glandular epithelium (SGE) next to the uterine lumen, deep glandular epithelium (DGE) next to the myometrium, and middle glandular epithelium (MGE) as the portion in between. Stromal cells were classified as follows: superficial and deep caruncular stroma (SCS, DCS) and superficial and deep intercaruncular stroma (SIS, DIS). The cell type to be studied was analyzed separately by interactively removing the others. Ten fields were analyzed and measured separately in each section of glandular epithelia and stromal cells. Luminal epithelium was not measured by image analysis since 10 fields could not be obtained in each slide. With the use of color-discrimination software, the total area of positively stained cells (brown reaction product) was measured and expressed as a ratio of the total area of cell nuclei (brown reaction product + blue hematoxylin).

2.4. Statistical analysis

The results from image analysis are presented as box and whisker plots where 50% of the values are within the box and the median is marked with a line. The bars represent the 5th and 95th percentiles. The ratio of positively stained area of the seven compartments in each uterine zone was analyzed by ANOVA on ranks (Kruskal-Wallis test) and specific comparisons against the control group were determined by Dunn's test (SigmaStat; SPSS Inc, Chicago, IL, USA).

3. RESULTS

3.1. General observations

Glandular epithelium in the deep glands (DGE) was stained more intensely than that closer to the uterine lumen (SGE) (Fig. 1a), and this feature was maintained in the treated groups. Caruncular stroma was more densely packed than intercaruncular stroma. Superficial stroma was more densely packed than deep stroma in both caruncular and intercaruncular endometrium. A negative

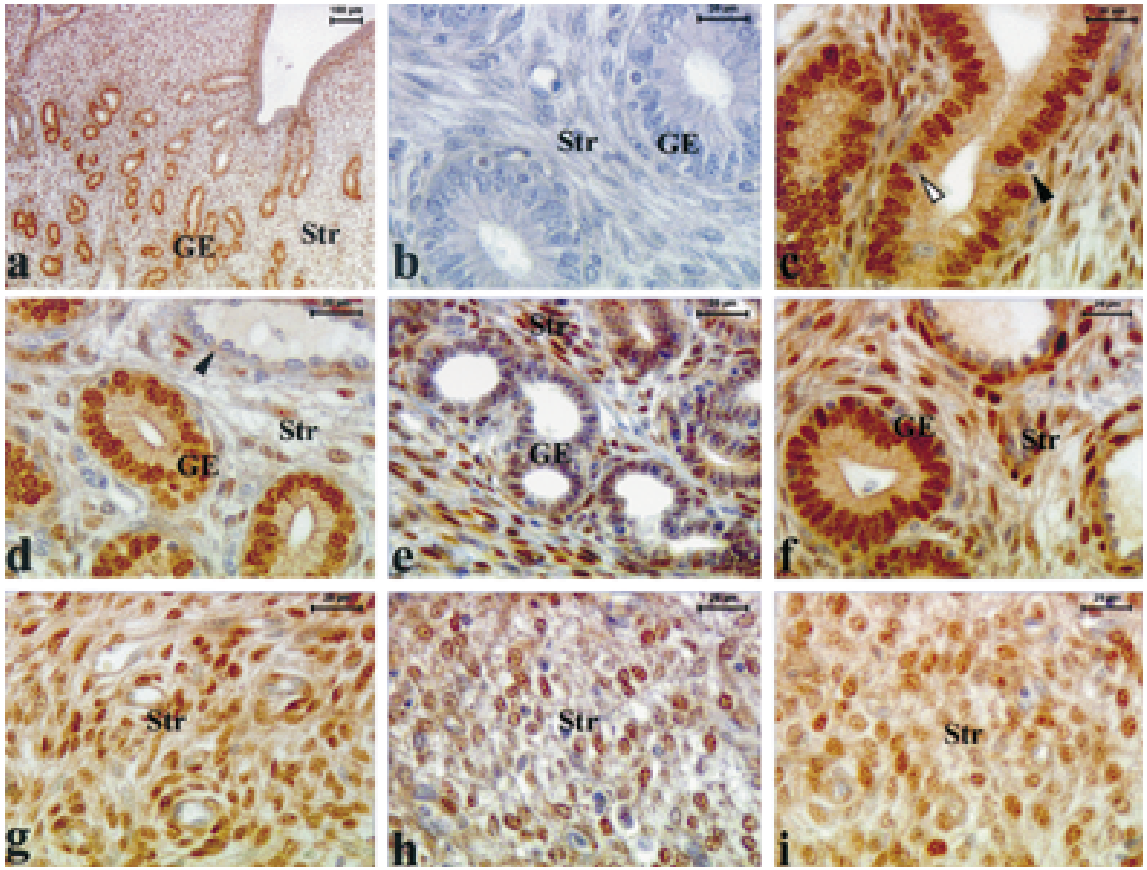

Figure 1. Immunohistochemical localization of ERα in endometrium of control and estradiol-treated lambs (GE = glandular epithelium, Str = stroma). (**a**) intercaruncular endometrium of a control lamb; (**b**) negative control; (**c**) intercaruncular endometrium of a control lamb (white arrow = columnar negative cell, black arrow = round-shaped negative cell); (**d**) intercaruncular endometrium of a control lamb (black arrow = endothelial cell); (**e**) intercaruncular endometrium of a lamb of group II; (**f**) intercaruncular endometrium of a lamb of group V; (**g**) caruncular endometrium of a control lamb; (**h**) caruncular endometrium of lamb of group II; (**i**) caruncular endometrium of a lamb of group V.

control is shown in Figure 1b. Staining for ERα was seen in the nuclei of epithelial and stromal cells. Two types of negative cells were seen in the glandular epithelium (Fig. 1c): columnar cells that reached to the lumen (white arrow) and round-shaped cells located close to the basal membrane (black arrow). Vascular endothelial cells were devoid of specific staining (Fig. 1d, black arrow). Positive ERα immunoreactivity was also observed in smooth muscle cells (not shown).

3.2. Epithelial cells

Figure 2 shows the image analysis score of positive ERα immunoreactivity in deep, middle and superficial glandular epithelium in upper, middle and lower uterine zones. A significant down regulation (Fig. 1e) occurred in all types of glandular epithelium of the lower uterine zone (Figs. 2g, 2h and 2i) and in DGE of the middle zone in lambs of Group II (Fig. 2d). Receptor expression was restored in most cell types 24 h after the first injection and the levels remained high during the rest of the experimental period (Figs. 1f and 2). No subsequent effects of repeated E2 injections could be seen. A tendency to a decrease in positive staining was also observed 12 h after the injection (Group II) in the rest of the glandular cells (Figs. 2a, 2b, 2c, 2e and 2f).

3.3. Stromal cells

The image analysis score of stroma was similar to that observed in the glandular

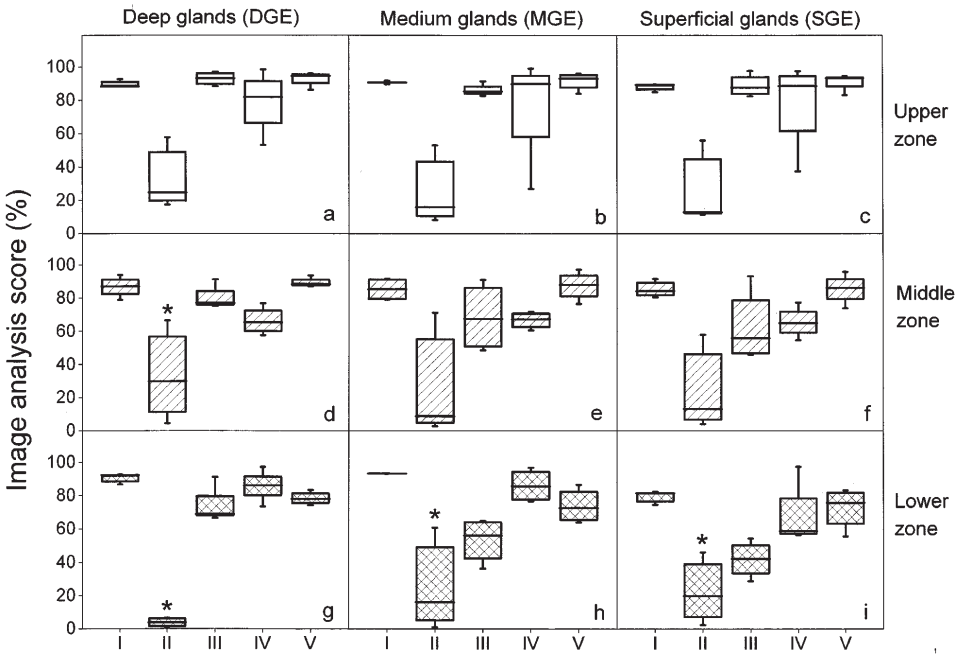

Figure 2. Image analysis score of positive ERα immunoreactivity in deep, middle and superficial glandular epithelium in upper, middle and lower uterine zones of control lambs (Group I), and lambs treated with one (Groups II and III), or two (Group IV), or three (Group V) estradiol (E2) injections. Animals were slaughtered 12 h (Group II) or 24 h (Groups III, IV and V) after the last injection. The upper zone is the portion of the uterus next to the oviduct, the lower zone is the portion of the uterus next to the cervix, and the middle zone the area in between. Values with asterisks are significantly different from Group I (controls), $P < 0.05$.

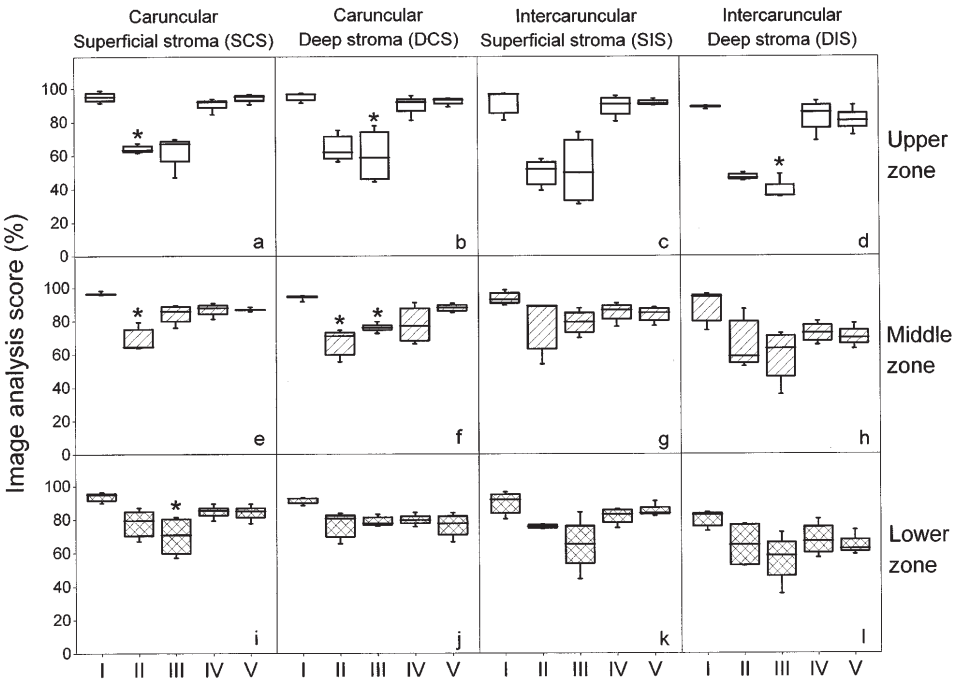

Figure 3. Image analysis score of positive ERα immunoreactivity in superficial and deep stroma in caruncular and intercaruncular endometrium in upper, middle and lower uterine zones of control lambs (Group I), and lambs treated with one (Groups II and III), or two (Group IV), or three (Group V) estradiol (E2) injections. Animals were slaughtered 12 h (Group II) or 24 h (Groups III, IV and V) after the last injection. The upper zone is the portion of the uterus next to the oviduct, the lower zone is the portion of the uterus next to the cervix, and the middle zone the area in between. Values with asterisks are significantly different from Group I (controls), $P < 0.05$.

epithelium (Fig. 3). Superficial and deep caruncular stroma (SCS, DCS) of the lambs of Group II and/or III had significantly less staining than control group (Figs. 3a, 3b, 3e, 3f and 3i), except for DCS in the lower uterine zone (Fig. 3j). Representative staining of stromal cells in the caruncular endometrium of lambs of Groups I, II and V are shown in Figures 1g, 1h and 1i respectively. Staining of stroma in the intercaruncular region differed significantly among groups only in the DIS of the upper uterine zone (Fig. 3d).

The down regulation in stromal cells was less pronounced than in epithelial cells; the average of the positive staining area in all stromal cells (considered both location and zone of the uterus) decreased from 87% (control) to 67% (12 h after the injection) while for the epithelial cells it was 92% to 27% respectively. The down regulation in stromal cells was maintained for a longer period (24 h in stromal cells vs. 12 h in epithelial cells) after the first E2 injection. Down regulation in the stroma was more prominent in the upper zone as compared to the middle and lower zones. In glandular epithelium, down regulation was most significant in the lower zone.

4. DISCUSSION

To our knowledge, this is the first immunohistochemical quantitative study of E2

regulation of ERα expression in ovine endometrium before ovarian cyclicity starts. In control lambs, the ratio of the total area of cell nuclei positively stained to ERα was almost 100%, confirming that the prepubertal ewe has high uterine ER concentrations [8, 15, 17]. This is consistent with reports on ER expression in ovariectomized and anestrous ewes [5, 27], and shows that also in noncyclic animals (low levels of ovarian steroids) the uterus has the receptors required to respond to estrogens.

An interesting and consistent finding in this study is that deep glandular epithelium was stained more intensely than superficial glandular epithelium, suggesting that the former cells are more sensitive to estrogen stimulation in the immature ewe. This is in agreement with previous studies in the cyclic ewe where the intensity of these cells were consistently higher [5], but different from another study [12] in the ovariectomized ewe where ER was absent in deep glandular epithelium but a moderate staining was found in superficial glandular epithelium. It was demonstrated in the rabbit, that cells at the bottom of the glands (deep glandular epithelium) proliferate and migrate towards the lumen after E2 treatment [7], but the role of these cells in the ewe and the reason why deep glandular epithelium express higher levels of ER remains to be elucidated.

The results show that E2 was able to down regulate ERα expression in epithelial and stromal cells of most of the uterine zones studied, but this effect was transitory and receptor expression was restored 12 h and/or 24 h after the injection. This is consistent with our previous results where ER determinations were done by binding assays and enzyme immunoassays [17]. Since a loss of immunoreactive ERα was described in our previous report [17] as well as in the present one, we can now confirm that the initial decrease in receptor concentration after E2 treatment was due to a loss of the protein itself, instead of a reduction in the binding capacity for the steroid. The decrease in ERα could be caused by an inhibition of receptor synthesis or by an increase in the action of specific proteases [1, 24]. The first cannot be explained by the ER mRNA levels, since mRNA expression was not decreased 12 h after the first injection [17]. On the other hand, a rapid and transient decrease (6 h) of ER mRNA after E2 treatment was reported in rats [24] and a reduction in the rate of translation can also be proposed. Besides, a decline in ER protein within 1 h after E2 treatment in the absence of transcription and protein synthesis was reported in the pituitary and it was concluded that the negative feedback loop of E2 on ER expression was nongenomic [1]. The ER down regulation found in the present experiment is different from the reported receptor up regulation induced by estrogen in cyclic and ovariectomized ewes [19, 23]. Though, it is in agreement with another report [12] that found a temporal decrease in ERα in some of the uterine cell compartments in the ovariectomized adult ewe after E2 injection. Likewise, ovariectomized ewes treated with subcutaneous implants of E2 for 12 days showed absent or weak staining in all uterine compartments, except in deep stroma [5]. The regulatory response to E2 on ER may depend on the initial endometrial expression of ERα (e.g., reproductive status: prepubertal, cyclic, anestrous or ovariectomized ewes) and on the dose and type of E2 treatment (infusions, implants or injections). The E2 dose used here (1 $\mu g \cdot kg^{-1}$) and in others studies on E2-regulation of steroid receptor expression [11, 12] resulted in supraphysiological levels [16], and the ERα down regulation observed may represent a mechanism to control the action of the hormone on the cell. The restoration of ERα immunostaining found in this study is consistent with the 2- to 3-fold increase in ERα mRNA as described previously [17].

The immunohistochemical technique allows to determine changes in the receptor presence in specific cell types. In this study, E2 regulation of ERα showed a similar pattern in epithelial and stromal cells,

but there were cell type specific differences in timing and strength of E2 action. The down regulation in stromal cells was less pronounced than in glandular epithelial cells, but was maintained for a longer period. These data suggest that the expression of ERα in stromal cells is not so tightly regulated by E2 as in epithelial cells. Similarly, ERα in epithelial cells of the reproductive tract in the neonatal mouse increased with age, but ERα immunostaining in stroma was almost constant with aging [30]. It has been demonstrated that steroid stimulation of the epithelial cells is also mediated by a receptor mechanism in the underlying stroma that produces factors acting on the epithelial cells [4, 6]. From our study we cannot address this problem, and the question if the higher ERα expression maintained in the stromal cells is responsible for the recovery of ERα expression in the epithelial cells still remains unanswered. Differences were also noted along the uterus; down regulation in the endometrial stroma was more prominent in the upper zone as compared to the middle and lower zones. This trend was opposite to what occurred in glandular epithelium, where down regulation was most significant in the cells of the lower zone.

In summary, the results of this study demonstrate that E2 regulation of endometrial ERα expression in lambs is biphasic in both stromal and epithelial cells, but the characteristics of the down regulation differ between cell types and endometrial depth locations.

ACKNOWLEDGMENTS

The authors want to thank H. Eriksson and E.G. Garófalo for constructive criticism of this manuscript. We also want to thank C. Tasende for cooperation in the experimental design and P. Rubianes for technical assistance. The present study received financial support from the Veterinary Faculty, University of Uruguay, the Swedish University of Agricultural Sciences, the Swedish Society for Medical Research and the Swedish Medical Research Council (Grant 03972).

REFERENCES

[1] Alarid E.T., Bakopoulos N., Solodin N., Proteasome-mediated proteolysis of estrogen receptor: A novel component in autologous down-regulation, Mol. Endocrinol. 13 (1999) 1522–1534.

[2] Bigsby R.M., Cunha G.R., Estrogen stimulation of deoxyribonucleic acid synthesis in uterine epithelial cells which lack estrogen receptors, Endocrinology 119 (1986) 390–396.

[3] Brenner R.M., West N.B., McClellan M.C., Estrogen and progestin receptors in the reproductive tract of male and female primates, Biol. Reprod. 42 (1990) 11–19.

[4] Buchanan D.L., Setiawan T., Lubahn D.B., Taylor J.A., Kurita T., Cunha G.R., Cooke P.S., Tissue compartment-specific estrogen receptor α participation in the mouse uterine epithelial secretory response, Endocrinology 140 (1999) 484–491.

[5] Cherny R.A., Salamonsen L.A., Findlay J.K., Immunocytochemical localization of oestrogen receptors in the endometrium of the ewe, Reprod. Fertil. Dev. 3 (1991) 321–331.

[6] Cooke P.S., Buchanan D.L., Yound P., Setiawan T., Brody J., Korach K.S., Taylor J., Lubahn D.B., Cunha G.R., Stromal estrogen receptors mediate mitogenic effects of estradiol on uterine epithelium, Proc. Natl. Acad. Sci. USA 94 (1997) 6535–6540.

[7] Conti C.J., Gimenez-Conti I.B., Conner E.A., Lehman J.M., Gerschenson L.E., Estrogen and progesterone regulation of proliferation, migration and loss in different target cells of rabbit uterine epithelium, Endocrinology 114 (1981) 345–351.

[8] Garófalo E.G., Tasende C., Uterine estrogen and progesterone receptors in prepubertal ewes: distribution in myometrium, endometrium and caruncles, Vet. Res. 27 (1996) 177–183.

[9] Gorski J., Hou Q., Embryonic Estrogen Receptors: Do they have a physiological function?, Environ. Health Perspect. 103 (1995) 69–72.

[10] Greco T.L., Duello T.M., Gorski J., Estrogen Receptors, Estradiol, and Diethylstilbestrol in Early Development: The Mouse as a Model for the Study of Estrogen Receptors and Estrogen Sensitivity in Embryonic Development of Male and Female Reproductive Tracts, Endocr. Rev. (1993) 59–71.

[11] Greiss F.C., Rose J.C, Kute T.E, Kelly R.T., Winkler L.S., Temporal and receptor correlates of the estrogen response in sheep, Am. J. Obstet. Gynecol. 154 (1986) 831–838.

[12] Ing N.H., Tornesi M.B., Estradiol up-regulates estrogen receptor and progesterone receptor gene expression in specific ovine uterine cells, Biol. Reprod. 56 (1997) 1205–1215.

[13] Lessey B.A., Wahawisan R., Gorell T.A., Hormonal regulation of cytoplasmic estrogen receptor and progesterone receptors in the beagle uterus and oviduct, Mol. Cell Endocrinol. 21 (1981) 171–180.

[14] Lubahn D.B., Mayer J.S., Golding T.S., Couse J.F., Korach K.S., Smithies O., Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene, Proc. Natl. Acad. Sci. USA 90 (1993) 11162–11166.

[15] Meikle A., Tasende C., Rodriguez M., Garófalo E.G., Effects of estradiol and progesterone on the reproductive tract and on uterine sex steroid receptors in female lambs, Theriogenology 48 (1997) 1105–1113.

[16] Meikle A., Tasende C., Garófalo E.G., Forsberg M., Priming effect of exogenous oestradiol on luteinizing hormone secretion in prepubertal lambs, Anim. Reprod. Sci. 54 (1998) 75–85.

[17] Meikle A., Forsberg M., Sahlin L., Masironi B., Tasende C., Rodríguez-Piñón M., Garófalo E.G., A biphasic action of estradiol on estrogen and progesterone receptor expression in the lamb uterus, Reprod. Nutr. Dev. 40 (2000) 283–293.

[18] Meikle A., Garófalo E.G., Rodríguez-Piñón M., Tasende C., Sahlin L., Regulation by gonadal steroids of estrogen and progesterone receptors along the reproductive tract in lambs, Acta Vet. Scand. (in press).

[19] Miller B.G., Murphy L., Stone G.M., Hormone receptor levels and hormone, RNA and protein metabolism in the genital tract during the oestrous cycle of the ewe, J. Endocrinol. 73 (1977) 91–98.

[20] Miller B.G., Wild J., Stone S.M., Effects of progesterone on the oestrogen-stimulated uterus: a comparative study of the mouse, guinea pig, rabbit and sheep, Aust. J. Biol. Sci. 32 (1979) 649–560.

[21] Quarmby V.E., Korach K.S., The influence of 17β-estradiol on patterns of cell division in the uterus, Endocrinology 114 (1984) 694–702.

[22] Quarmby V.E., Korach K.S., Differential regulation of protein synthesis by estradiol in uterine compartment tissues, Endocrinology 115 (1984) 687–697.

[23] Rexroad C.E. Jr., Estrogen and progestogen binding in the myometrium of the ewe. II. Regulation by estradiol and progesterone, J. Anim. Sci. 53 (1981) 1070–1076.

[24] Sahlin L., Norstedt G., Eriksson H., Estrogen regulation of the estrogen receptor and insuline-like growth factor-I in the rat uterus: a potential coupling between effects of estrogen and IGF-I, Steroids 59 (1994) 421–430.

[25] Sirbasku D.A., Benson R.H., Estrogen-inducible growth factors that may act as mediators (estromedins) of estrogen-promoted tumor cell growth, in: Sato G.S., Ross R. (Eds.), Hormones and cell culture, Cold Spring Harbor NY, 1979, pp. 477–490.

[26] Stone G.M., McCaffery C., Miller B.G., Effects of progesterone on nuclear and cytosol steroid receptor levels in the oestrogen-stimulated uterus: comparison of the sheep and mouse, Aust. J. Biol. Sci. 35 (1982) 403–415.

[27] Tasende C., Rodríguez-Piñón M., Meikle A., Forsberg M., Garófalo E.G., Expression of estrogen and progesterone receptors in the pituitary gland and the uterus of anestrous ewes after treatment with progesterone and GnRH, Abstract accepted, World Buiatrics Congress, December 2000.

[28] Tibbets T.A., Mendoza-Meneses M., O'Malley B.W., Conneely O.M., Mutual and Intercompartmental regulation of estrogen receptor and progesterone receptor expression in the mouse uterus, Biol. Reprod. 59 (1998) 1143–1152.

[29] Wang H., Masironi B., Eriksson H., Sahlin L., A comparative study of estrogen receptors α and β in the rat uterus, Biol. Reprod. 61 (1999) 955–964.

[30] Yamashita S., Newbold R.R., McLachlan J.A., Korach K.S., Developmental pattern of estrogen receptor expression in female mouse genital tracts, Endocrinology 125 (1989) 2888–2896.

[31] Zhao Y., Williams L.M., Hannah L.T., Ross A.W., McKelvey W.A.C., Robinson J.J., Oestrogen and progesterone receptor immunoreactivity and c-fos expression in the ovine cervix, J. Reprod. Fertil. 115 (1999) 287–292.