Histol Histopathol (2007) 22: 169-176

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Steroid receptors ERα, ERß, PR-A and PR-B are differentially expressed in normal and atrophic human endometrium

I. Mylonas, U. Jeschke, N. Shabani, C. Kuhn, S. Kunze, D. Dian, C. Friedl, M.S. Kupka and K. Friese

1st Department of Obstetrics and Gynaecology, Ludwig-Maximilians, University Munich, Germany

Summary. Objective: The endometrium expresses estrogen (ER) and progesterone receptor (PR), which are related to autocrine and paracrine processes that respond to estrogen and progesterone. Therefore, the aim of this study was to evaluate the distribution pattern of ERα, ERß, PR-A and PR-B with monoclonal antibodies in normal human endometrial tissue. Study Design: Human endometrial tissue was obtained from 84 premenopausal and 11 postmenopausal patients and immunohistochemically analysed with monoclonal antibodies against ERα, ERß, PR-A and PR-B. Results: ERα, PR-A and PR-B declined significantly ($p<0.001$, $p<0.05$, $p<0.05$ respectively) in glandular epithelium from proliferative to late secretory phase. The ERß immunohistochemical reaction showed a similar significant declining pattern ($p<0.05$), although the staining intensity was lower than that of ERα. While ERα, ERß and PR-B decrease significantly in atrophic endometrial tissue compared to proliferative endometrium, a significant up-regulation of PR-A was observed compared to late secretory phase ($p<0.05$). Conclusion: ERα, ERß, PR-A and PR-B were expressed in normal human endometrium with a cyclical variation during the menstrual cycle. In normal postmenopausal endometrial tissue, a down-regulation of ERα, ERß and PR-B occurs with a subsequent higher expression of PR-A. These results show the presence of steroid receptors in human epithelium, indicating that these cells respond to estrogen and progesterone, thus playing a significant role in endometrial physiology.

Key words: Endometrium, Immunohistochemistry, Estrogen receptor alpha (ERα), Estrogen receptor beta (ERß) progesterone receptor A (PR-A), Progesterone receptor B (PR-B)

Offprint requests to: Dr. Udo Jeschke, PhD, 1st Department of Obstetrics and Gynecology, Ludwig Maximilians University Munich, Maistrasse 11, 80337 Munich, Germany. e-mail: udo.jeschke@med.uni-muenchen.de

I.M., U.J. and N.S. contributed equally to this work

Introduction

The endometrium is one of the most dynamic tissues in humans, undergoing proliferative and secretory changes that are thought to be primarily controlled by the ovarian steroid hormones estrogen and progesterone. These hormones in human endometrium are thought to act through the estrogen receptor (ER) and progesterone receptor (PR) (Snijders et al., 1992). In several studies both receptors were analysed in paraffin-fixed normal human endometrial tissue (Garcia et al., 1988; Lessey et al., 1988; Snijders et al., 1992; Jones et al., 1995; Mylonas et al., 2004a, 2005) and isolated glandular epithelial cells (Mylonas et al., 2000) using immunohistochemical assays.

For several years it was generally believed that only single ER and PR receptors existed. However, the discovery of a new ER with specificity for estrogens has created new insights in the estrogen signalling system (Kuiper et al., 1998). The novel receptor ERß is highly homologous to the classical ER (ERα), which can bind estradiol with a high affinity and stimulate transcription of an ER target gene (Kuiper et al., 1997). Additionally, PR is expressed as two major isoforms, PR-A and PR-B, which arise from alternative transcriptional starting sites within the same gene. PR-A encodes 769 amino acids and lacks the first 164 amino acids of the N-terminus of the PR-B (933 amino acids) (Horwitz et al., 1983; Krett et al., 1988; Graham et al., 1995; Graham and Clarke, 2002). Although PR-A and PR-B were thought to occur in similar amounts, it is now clear that they are differentially expressed and thus have distinct functions in several human tissues, including human endometrium (Graham et al., 1995; Leslie et al., 1997; Kumar et al., 1998; Graham and Clarke 2002).

Although ER and PR can be measured in paraffin-fixed normal human endometrial tissue (Garcia et al., 1988; Lessey et al., 1988; Snijders et al., 1992; Jones et al., 1995; Mylonas et al., 2004a, 2005), there were several difficulties in the visualisation of ERß and PR-B

due to the lack of appropriate monoclonal antibodies. Recently, the immunohistochemical expression of ERß in human endometrium was demonstrated using polyclonal (Lecce et al., 2001) and monoclonal antibodies (Mylonas et al., 2004a, 2005; Taylor et al., 2005). However, several conflicting data and issues regarding the expression of ERß in human endometrial tissue still remain. For the PR, it is now clear that commercially available antibodies against PR might not have equal affinity to PR-A and PR-B, especially using immunohistochemistry. Since PR-B is not recognized by many used antibodies, the possibility of an under-reporting PR-B expression should be considered (Mote et al., 2001).

Therefore, the aims of this study were the evaluation of the distribution patterns of ERα, ERß and PR-A and PR-B using recently available commercial monoclonal antibodies in normal human pre- and postmenopausal endometrial tissue.

Materials and methods

Tissue samples

Samples of normal human endometrium were obtained from 84 premenopausal, non-pregnant patients undergoing gynaecological surgery either by D&C or hysterectomy for benign diseases. All pathological endometria were excluded from this study. Endometrium samples were classified according to anamnestical and histological dating (Dallenbach-Hellweg and Poulsen, 1985; Kurman 2002) into proliferative (day 1-14, n=46), early secretory (day 15-22, n=18) and late secretory phase (day 23-28, n =20) as previously described (Jones et al., 1995; Mylonas et al., 2000). Additionally, eleven (n=11) samples of atrophic human endometrial tissue from postmenopausal patients with no hormonal substitute that underwent hysterectomy or D&C for benign diseases, mainly urogenitary prolaps, were analysed.

Immunohistochemistry

Immunohistochemistry was performed using a combination of microwave-oven heating and the standard streptavidin-biotin-peroxidase complex using the mouse-IgG-Vectastain Elite ABC kit (Vector Laboratories, Burlingame, California, USA) as previously described (Mylonas et al., 2004a, 2005). Mouse monoclonal antibodies used for the experiments are listed in Table 1. For positive controls sections of human breast cancer tissue and normal colon were used, while human ileum served as negative control tissue.

Briefly, paraffin-fixed tissue sections were dewaxed using xylol for 15min, rehydrated in an alcohol row, and subjected to antigen retrieval on a high setting for 10min in a pressure cooker in sodium citrate buffer (pH 6.0), containing citrate acid 0.1M and sodium citrate 0.1M in distillate water. After cooling, the slides were washed twice in PBS. Endogenous peroxidase activity was quenched by immersion in 3% hydrogen peroxide (Merck, Darmstadt, Germany) in methanol for 20 min. Non-specific binding of the primary antibodies was blocked by incubating the sections with diluted normal serum (10ml PBS with 150μl horse serum, provided by Vectastain Elite ABC kit) for 20min at room temperature. Sections were then incubated at room temperature for 60min with the primary antibodies. ERα and PR-A were diluted in dilution-medium (Dako, Glostrup, Denmark), while ERß and PR-B were diluted in PBS. After washing with PBS, the slides were incubated in diluted biotinylated serum for another 30 min at room temperature (10 ml PBS, 50μl horse serum, provided by Vectastain Elite ABC kit). After incubation with the avidin-biotin peroxidase complex (diluted in 10ml PBS, reagent ABC provided by Vectastain Elite ABC kit) for another 30 min and a repeated washing step with PBS, visualisation was performed with substrate and chromagen 3,3'-diaminobenzidine (DAB; Dako, Glostrup, Denmark) for 8-10min. The slides where counterstained further with Mayer's acidic hematoxylin and washed in an alcohol multiple-row (50-98%). After xylol treatment the slides were covered. Negative controls were performed by replacing the primary antibody with normal mouse serum. Positive controls for ERα, PR-A and PR-B include human invasive breast cancer. The ERß antibody was tested positive on human colon tissue. Positive cells showed a brownish colour and negative controls as well as unstained cells were blue.

Evaluation and statistical analysis

The intensity and distribution of the specific

Table 1. Antibodies used for immunohistochemical characterization of endometrial glandular cells.

Antibody	Clone	Isotype	Dilution	Source
Erα	1D5	mouse IgG1	1:150	Immunotech, Hamburg, Germany
ERß	PPG5/10	mouse IgG2a	1:50	Serotec, Oxford, United Kingdom
PR-A	10A9	mouse IgG2a	1:50	Immunotech, Hamburg, Germany
PR-B	SAN27	mouse IgG1	1:50	NovoCastra, Newcastle, United Kingdom

ER: estrogen receptor, PR: progesterone receptor.

immunohistochemical staining reaction was evaluated using a semi-quantitative method (IRS-score) as previously described (Remmele and Stegner, 1987) and previously used in the evaluation of inhibin/activin subunits (Mylonas et al., 2004b, 2006), CA-125 (Mylonas et al., 2003b) as well as endometrial steroid receptor expression (Mylonas et al., 2000, 2004a, 2005).

The slides were examined by two independent observers, including a gynaecological pathologist (N.S.). Since stromal cells expressed also these steroid receptors but with a very variable intensity, only glandular epithelial cells were evaluated for statistical analysis. At least 3 representative fields between the endometrial basalis layer and luminal epithelium were selected and approximately 200 cells of these areas were counted. In 32 cases of the 369 evaluated slides (8,67%) there was a discrepancy between the two observers and these slides were again evaluated by both together. The level of agreement between the two observers was 91,33%.

The IRS score was calculated as follows: IRS=SIxPP, where SI is the optical stain intensity (graded as 0 = no, 1 = weak, 2 = moderate, and 3 = strong staining) and PP the percentage of positive stained cells. The PP was estimated by counting out approximately 200 cells and it was defined as 0 = no staining, 1 = <10%, 2 = 11-50%, 3 = 51-80%, and 4 = >81%. The Mann-Whitney rank-sum test was used to compare the means of the different IRS scores (SPSS, Chicago, IL, USA). Significance was assumed at $p<0.05$.

Results

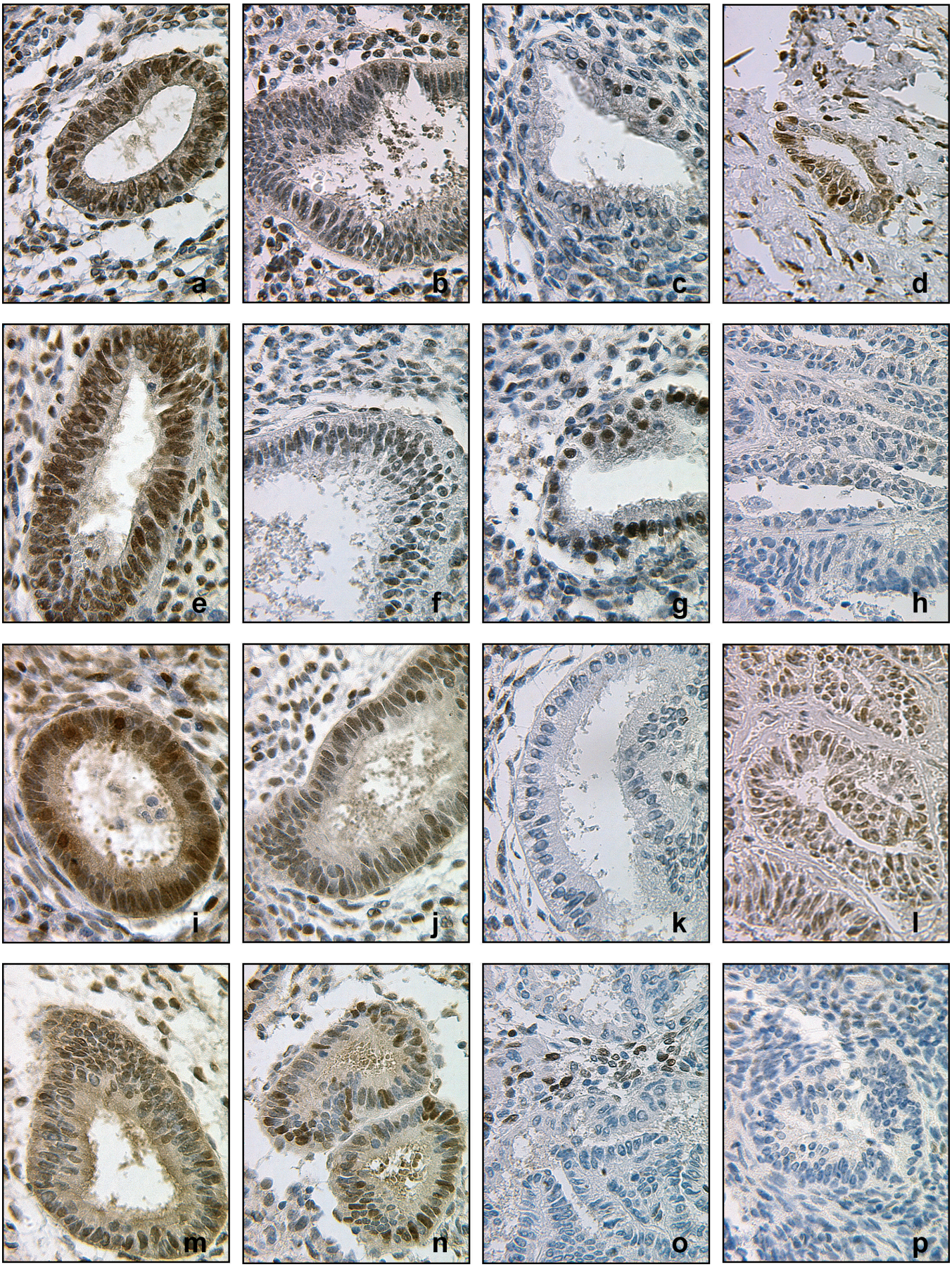
Both glandular epithelial and stromal cells demonstrated nuclear immunoreactivity for Erα, ERß, PR-A and PR-B, confirming previous results (Garcia et al., 1988; Lessey et al., 1988; Snijders et al., 1992; Jones et al., 1995; Mylonas et al., 2004a, 2005; Leslie et al., 2005). While the staining reaction of ERα in biopsies in the proliferative phase was intense (Fig. 1a), it decreased continuously from early (Fig. 1b) till late secretory phase (Fig. 1c) confirming previous results (Garcia et al., 1988; Snijders et al., 1992; Jones et al., 1995; Mylonas et al., 2004a, 2005). Atrophic endometrial tissue also expressed ERα, but with a lower intensity than proliferative endometrial tissue (Fig. 1d). ERß immunostaining patterns were also expressed in endometrial glandular and stromal cells. However, ERß immunostaining intensity was lower compared to the Erα staining reaction. The intensity of positive reacted epithelial cells was higher in the proliferative phase (Fig. 1e) decreasing in early (Fig. 1f) and in late secretory phase (Fig. 1g) during the menstrual cycle. ERß was also expressed in atrophic endometrium with the weakest immunolabelling intensity compared to premenopausal endometrial tissue (Fig. 1h). Interestingly, stromal cells expressed immunohistochemically both ER with a variable intensity.

The PR-A immunohistochemical reaction showed a similar declining immunolabelling between proliferative (Fig. 1i) and early secretory phase (Fig. 1j) compared to late secretory phase (Fig. 1k), while it was also expressed in stromal cells. Atrophic endometrium showed also a PR-A immunolabelling being more intense compared to late secretory phase (Fig. 1l). PR-B was also demonstrated in both premenopausal and postmenopausal endometrial tissue. While the immunolabelling was intense during proliferative (Fig. 1m) and early secretory phase (Fig. 1n), decreasing during late secretory phase (Fig. 1o) with the ongoing of the menstrual cycle, reaching a weak immunostaining in atrophic endometrium (Fig. 1p). Interestingly, stromal cells also expressed the PR-B, but with a variable pattern. The immunolabelling intensity for both PR receptors was, in contrast to ER, similar.

The IRS score for the ERα declined significantly ($p<0.001$) in the glandular epithelium from proliferative to late secretory phase. The intensity score for ERß also declined in the glandular epithelium from proliferative to early ($p<0.05$) and late secretory phase ($p<0.05$) (Fig. 2). The PR-A expression also decreased significantly between proliferative and late secretory phase ($p<0.001$) and early secretory and late secretory phase ($p<0.05$). PR-B showed a similar decline between proliferative and early secretory compared to late secretory phase ($p<0.05$ each) (Fig. 2).

Atrophic tissue demonstrated the lowest immunolabelling expression for ERß and PR-B compared to premenopausal endometrium. The immunostaining for ERα was similar to late secretory phase. Interestingly, the PR-A expression was significantly higher in atrophic endometrium compared to late secretory phase ($p<0.05$). The IRS score showed a significant decline from proliferative phase to atrophic endometrium for ERα ($p<0.05$), ERß ($p<0.01$) and PR-B ($p<0.05$). Additionally, PR-B was also significantly

Fig. 1.a-p. Immunohistochemical expression of ERα, ERß and PR-A, PR-B. While the staining reaction of ERα in the proliferative phase was intense (**a**), it decreased continuously from early (**b**) till late secretory phase (**c**). Atrophic endometrial tissue expressed ERα with a lower intensity than proliferative endometrial tissue (**d**). The intensity of positive reacted epithelial cells against ERß antibody was higher in the proliferative phase (**e**) decreasing in early (**f**) and in late secretory phase (**g**) during the menstrual cycle. ERß was also expressed in atrophic endometrium with the weakest immunolabelling intensity compared to premenopausal endometrial tissue (**h**). The PR-A immunohistochemical reaction showed a similar declining immunolabelling between proliferative (**i**) and early secretory phase (**j**) compared to late secretory phase (**k**), while it was also expressed in stromal cells. Atrophic endometrium also showed also a PR-A immunolabelling being more intense compared to late secretory phase (**l**). While the immunolabelling of PR-B was intense during the proliferative (**m**) and early secretory phase (**n**), it decreased during late secretory phase (**o**), reaching a weak immunostaining in atrophic endometrium (**p**). Interestingly, stromal cells also expressed the PR-B, but with a variable pattern. a-p, x 250


a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p

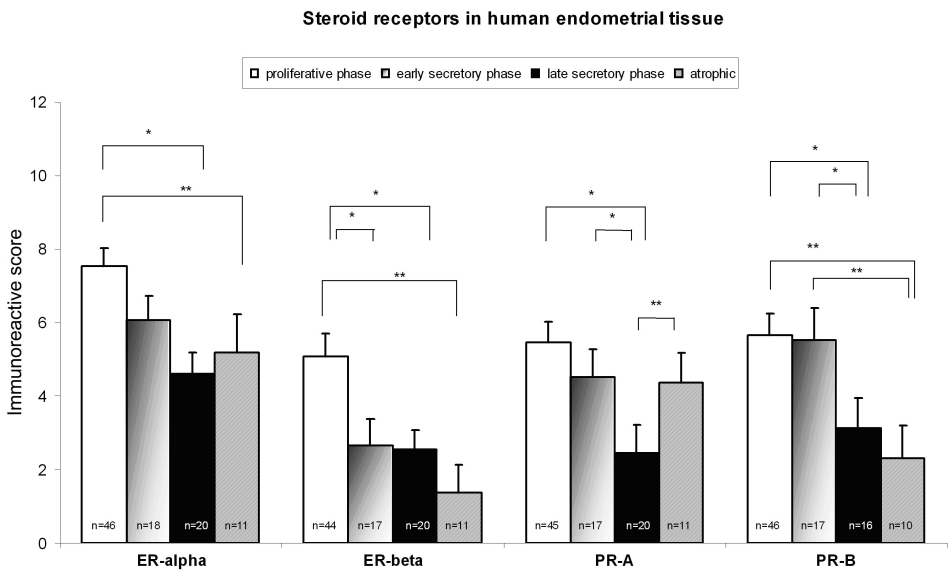


Fig. 2. IRS-score for ERa, ERß, PR-A and PR-B. The IRS score for ERa and ERß declined significantly ($p<0.001$ and $p<0.05$ respectively) in the glandular epithelium from proliferative to late secretory phase (one asterisk). Additionally, ERß expression was higher during proliferative compared to early secretory phase ($p<0.05$) (one asterisk). PR-A and PR-B reached the lowest significant expression in late secretory phase compared to samples from proliferative phase ($p<0.001$ and $p<0.05$ respectively, one asterisk) and early secretory compared to late secretory phase ($p<0.05$ each, one asterisk). ERα and ERß immunolabelling was similar to secretory endometria, but significantly lower compared to samples from the proliferative phase ($p<0.05$ and $p<0.01$ respectively). Atrophic endometrium showed a similar PR-A expression with proliferative endometrial tissue, being significantly higher than late secretory endometrium ($p<0.05$) (two asterisks). The PR-B expression also decreased significantly between proliferative and early secretory phase compared to atrophic endometrium ($p<0.05$ each) (two asterisks).

higher during early secretory phase than atrophic tissue ($p<0.05$) (Fig. 2).

Discussion

The endometrium is one principal target tissue of the pituitary-gonadal axis, but has also been recognized as an endocrine organ itself. The knowledge of the distribution pattern of steroid receptors might contribute to the understanding of steroid hormone function in human endometrium. Additionally, the newly detected steroid receptors ERß and PR-B and their characterisation in human endometrial tissue might elucidate several "paradox" findings that cannot be explained with the classical two receptor explanation. As an characteristic example of such contradictory findings of the antiestrogenic and partially estrogenic effect of tamoxifen (an antiestrogen widely used in the treatment of breast cancer) should be mentioned. Meanwhile, it is believed that tamoxifen exerts its antiestrogenic effects through the ERα, while its estrogenic potential is mediated through ERß (Kuiper et al., 1997; Peach et al., 1997; Mylonas et al., 2003a).

We demonstrated the expression of ERα, ERß, PR-A and PR-B in normal human endometrial tissue. However, ERß immunolabelling is less intense than that of ERα. There have been several studies measuring ER and PR in the human endometrium using immunohistochemical assays. Our results confirm the cyclical variations of ER and PR as described by several authors (Garcia et al., 1988; Snijders et al., 1992; Jones et al., 1995; Mylonas et al., 2004a, 2005). These immunohistochemical results show the presence of steroid receptors in human epithelium, indicating that these cells respond to estrogen and progesterone (Snijders et al., 1992). ER concentrations are maximal in the proliferative phase and decline in both the glandular and stromal compartment in the secretory phase of the cycle (Fujishita et al., 1997). The ER expression and distribution pattern might play an important role in normal endometrial function and pathogenesis and the expression and relationship of these steroid receptors could be of essential clinical implications. The presence and distribution pattern of ERß in endometrial epithelial cells confirms several previous reports (Rey et al., 1998; Matsuzaki et al., 1999; Lecce et al., 2001; Mylonas et al., 2004a, 2005) but not with the results of some other research groups (Critchley et al., 2002; Taylor et al., 2005). Our results indicate that glandular ERß expression predominantly occurs during the proliferative phase, declining as the menstrual phase continues. Differences in tissue distribution of ERα and ERß during the menstrual cycle suggest a substantial role in the modulation and function of estrogen activity in human endometrial tissue. ERß could have a different action and activity compared to ERα, since it exerts opposite transcriptional effects after binding to estrogens and antiestrogens than its ERα counterpart (Paech et al., 1997). Therefore, a synchronized expression of ERα and ERß seems to be essential to estrogen-related transcriptional activity in target organs. Differences in the ERα/ERß ratio could have important functional implications, probably as a result of the different binding characteristics (Kuiper et al., 1997). In addition, a disrupted expression of ERα and ß might play important roles in endometrial pathogenesis and carcinogenesis

(Fujimoto et al., 2002). Whether the ERß expression is influenced by PR-A or PR-B and/or progesterone remains to be elucidated.

During the proliferative phase, endometrial estradiol concentrations are high with a high tissue to plasma ratio (Alsbach et al., 1983). The high steroid receptor expression in glandular epithelium during the proliferative phase probably results from increased local estradiol concentration (Snijders et al., 1992). The significant decrease of ER in glandular epithelium is consistent with a drop in endometrial concentrations (Alsbach et al., 1983). Therefore, cyclical sex steroid hormone expressions are associated with the known changes of their receptors in endometrium. Additionally, since the transcription of the PR gene is induced by estrogens and inhibited by progesterone, the expression of ER and PR are thought to be closely associated (Savouret et al., 1994a,b). Meanwhile, it is generally accepted that estradiol up-regulates ER and PR, while progesterone down-regulates both receptors (Lessey et al., 1988). Recently, a significant decline of ERα was demonstrated in glandular epithelial cells after stimulation with tamoxifen, an antiestrogen, and the phytoestrogen genistein, whereas PR increased significantly after stimulation with both substances, suggesting also a functional association between these two steroid receptors (Mylonas et al., 2003a).

Concentrations of PR in the glandular epithelium are also high in the proliferative phase under the influence of estrogen and decrease after ovulation due to an increase of progesterone production. In contrast, no significant variations in endometrial stromal PR content throughout the menstrual cycle have been observed (Garcia et al., 1988; Snijders et al., 1992). Both PR-isoforms have been demonstrated in human endometrium and relative variations of PR-A/PR-B expression level during the menstrual cycle have been observed (Mangal et al., 1997; Wang et al., 1998). The ratio of PR-A to PR-B changes during the menstrual cycle, although the concentrations of PR-A remain constant and are higher compared to PR-B (Mangal et al., 1997). The relative amounts of PR-B increase during the proliferative phase reaching the highest levels during periovulatory phase. Thereafter, PR-B levels decrease rapidly and are almost undetectable at the end of the cycle (Mangal et al., 1997). We were able to demonstrate immunohistochemically a higher PR-B concentration during the proliferative and early secretory phase, which decreases significantly in the late secretory phase. The unique N-termini of PR-A and PR-B results in a different functionality. So PR-B is a stronger transcriptional activator of many genes compared to PR-A (Leslie et al., 1997; Jacobsen et al., 2005), but PR-A counters estrogen action directly by inhibiting ER function in a dominant-negative way (Vegeto et al., 1993). Interestingly, both PR isoforms can enhance differentiation in endometrial cancer cell, with PR-A inducing cell senescence and PR-B inducing a secretory phenotype (Dai et al., 2002). In PR-A knockout mice model the PR-B isoform induces cell growth (Mulac-Jericevic et al., 2000). Mice deficient of both PR's or only PR-A demonstrate endometrial proliferation suggesting endometrial growth through PR-B in the absence of PR-A (Lydon et al., 1995).

Postmenopausal endometrium also expressed ERα, ERß, PR-A and PR-B, but with a lower intensity compared to proliferative endometria. In atrophic endometrial tissue we observed, as expected, a down-regulation of ERα and ERß compared to proliferative endometrium, probably due to the decreased production of estradiol in the postmenopause. While PR-B decrease significantly in atrophic endometrial tissue, an up-regulation of PR-A was observed compared to late secretory endometrial tissue. Interestingly, ERα showed a significant decline with tamoxifen, an antiestrogen and genistein, a phytoestrogen, whereas PR expression increased significantly (Mylonas et al., 2003a). Therefore, we speculate that normal postmenopausal endometrial tissue is marked by a down-regulation of ERα, ERß and PR-B but with a subsequent higher expression of PR-A. Thus, the PR-A/PR-B ratio might play an important role in endometrial transission and subsequently influence endometrial pathogenesis.

In conclusion, we demonstrated ERα, ERß and PR-A and PR-B expression in normal human endometrium with a cyclical variation regarding all four steroid receptors during the menstrual cycle. These results show the presence of steroid receptors in human epithelium, indicating that these cells respond to estrogen and progesterone, mediating a significant role in endometrial physiology.

Acknowledgemetns. We would like to thank Mrs. S. Schulze (Munich) for their excellent work with the endometrial samples and Dr. D. Mayr, Dr. W. Sigg and Dr. S. Kriegel (Munich) for their support.

References

Alsbach G.P., Franck E.R., Poortman J. and Thijssen J.H. (1983). Subcellular distribution of estradiol and estrone in human endometrium and myometrium during the menstrual cycle. Contraception 27, 409-421

Critchley H.O., Henderson T.A., Kelly R.W., Scobie G.S., Evans L.R., Groome N.P. and Saunders P.T. (2002). Wild-type estrogen receptor (ERbeta1) and the splice variant (ERbetacx/beta2) are both expressed within the human endometrium throughout the normal menstrual cycle. J. Clin. Endocrinol. Metab. 87, 5265-5273.

Dai D., Wolf D.M., Litman E.S., White M.J. and Leslie K.K. (2002). Progesterone inhibits human endometrial cancer cell growth and invasiveness: down-regulation of cellular adhesion molecules through progesterone B receptors. Cancer Res. 62, 881-886.

Dallenbach-Hellweg G. and Poulsen H. (1985). Atlas der Histopathologie des Endometriums. Berlin, Heidelberg, New York, Springer.

Fujimoto J., Sakaguchi H., Aoki I., Toyoki H. and Tamaya T. (2002). Clinical implications of the expression of estrogen receptor-alpha and -beta in primary and metastatic lesions of uterine endometrial

cancers. Oncology 62, 269-277.

Fujishita A., Nakane P.K., Koji T., Masuzaki H., Chavez R.O., Yamabe T. and Ishimaru T. (1997). Expression of estrogen and progesterone receptors in endometrium and peritoneal endometriosis: an immunohistochemical and in situ hybridization study. Fertil. Steril. 67, 856-864.

Garcia E., Bouchard P., De Brux J., Berdah J., Frydman R., Schaison G., Milgrom E. and Perrot-Applanat M. (1988). Use of immunocytochemistry of progesterone and estrogen receptors for endometrial dating. J. Clin. Endocrinol. Metab. 67, 80-87.

Graham J.D., Yeates C., Balleine R.L., Harvey S.S., Milliken J.S., Bilous A.M. and Clarke C.L. (1995). Characterization of progesterone receptor A and B expression in human breast cancer. Cancer. Res. 55, 5063-5068.

Graham J.D. and Clarke C.L. (2002). Expression and transcriptional activity of progesterone receptor A and progesterone receptor B in mammalian cells. Breast Cancer Res. 4, 187-190.

Horwitz K.B., Mockus M.B., Pike A.W., Fennessey P.V. and Sheridan R.L. (1983). Progesterone receptor replenishment in T47D human breast cancer cells. Roles of protein synthesis and hormone metabolism. J. Biol. Chem. 258, 7603-7610.

Jacobsen B.M., Schittone S.A., Richer J.K. and Horwitz K.B. (2005). Progesterone-independent effects of human progesterone receptors (PRs) in estrogen receptor-positive breast cancer: PR isoform-specific gene regulation and tumor biology. Mol. Endocrinol. 19, 574-587.

Jones R.K., Bulmer J.N. and Searle R.F. (1995). Immunohistochemical characterization of proliferation, oestrogen receptor and progesterone receptor expression in endometriosis: comparison of eutopic and ectopic endometrium with normal cycling endometrium. Hum. Reprod. 10, 3272-3279.

Krett N.L., Wei L.L., Francis M.D., Nordeen S.K., Gordon D.F., Wood W.M. and Horwitz K.B. (1988). Human progesterone A-receptors can be synthesized intracellularly and are biologically functional. Biochem. Biophys. Res. Commun. 157, 278-285.

Kuiper G.G., Carlsson B., Grandien K., Enmark E., Haggblad J., Nilsson S. and Gustafsson J.A. (1997). Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138, 863-870.

Kuiper G.G., Shughrue P.J., Merchenthaler I. and Gustafsson J.A. (1998). The estrogen receptor beta subtype: a novel mediator of estrogen action in neuroendocrine systems. Front. Neuroendocrinol. 19, 253-286.

Kumar N.S., Richer J., Owen G., Litman E., Horwitz K.B. and Leslie K.K. (1998). Selective down-regulation of progesterone receptor isoform B in poorly differentiated human endometrial cancer cells: implications for unopposed estrogen action. Cancer Res. 58, 1860-1865.

Kurman R.J. (2002) Blaustein's pathology of the femal genital tract., 5th ed. New York, USA, Springer.

Lecce G., Meduri G., Ancelin M., Bergeron C. and Perrot-Applanat M. (2001). Presence of Estrogen Receptor beta in the Human Endometrium through the Cycle: Expression in Glandular, Stromal, and Vascular Cells. J. Clin. Endocrinol. Metab. 86, 1379-1386.

Leslie K.K., Kumar N.S., Richer J., Owen G., Takimoto G., Horwitz K.B. and Lange C. (1997). Differential expression of the A and B isoforms of progesterone receptor in human endometrial cancer cells. Only progesterone receptor B is induced by estrogen and associated with strong transcriptional activation. Ann. N.Y. Acad. Sci. 828, 17-26.

Leslie K.K., Stein M.P., Kumar N.S., Dai D., Stephens J., Wandinger-Ness A. and Glueck D.H. (2005). Progesterone receptor isoform identification and subcellular localization in endometrial cancer. Gynecol. Oncol. 96, 32-41.

Lessey B.A., Killam A.P., Metzger D.A., Haney A.F., Greene G.L. and McCarty K.S. Jr (1988). Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. J. Clin. Endocrinol. Metab. 67, 334-340.

Lydon J.P., DeMayo F.J., Funk C.R., Mani S.K., Hughes A.R., Montgomery C.A. Jr, Shyamala G., Conneely O.M. and O'Malley B.W. (1995). Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. Genes Dev. 9, 2266-2278.

Mangal R.K., Wiehle R.D., Poindexter A.N. 3rd and Weigel N.L. (1997). Differential expression of uterine progesterone receptor forms A and B during the menstrual cycle. J. Steroid Biochem. Mol. Biol. 63, 195-202.

Matsuzaki S., Fukaya T., Suzuki T., Murakami T., Sasano H. and Yajima A. (1999). Oestrogen receptor alpha and beta mRNA expression in human endometrium throughout the menstrual cycle. Mol. Hum. Reprod. 5, 559-564.

Mote P.A., Johnston J.F., Manninen T., Tuohimaa P. and Clarke C.L. (2001). Detection of progesterone receptor forms A and B by immunohistochemical analysis. J. Clin. Pathol. 54, 624-630.

Mulac-Jericevic B., Mullinax R.A., DeMayo F.J., Lydon J.P. and Conneely O.M. (2000). Subgroup of reproductive functions of progesterone mediated by progesterone receptor-B isoform. Science 289, 1751-1754.

Mylonas I., Speer R., Makovitzky J., Richter D.U., Briese V., Jeschke U. and Friese K. (2000). Immunohistochemical analysis of steroid receptors and glycodelin A (PP14) in isolated glandular epithelial cells of normal human endometrium. Histochem. Cell Biol. 114, 405-411.

Mylonas I., Jeschke U., Makovitzky J., Winkler L., Richter D.U., Friese K. and Briese V. (2003a). Immunohistochemical expression of steroid receptors and glycodelin A in isolated proliferative human endometrial glandular cells after stimulation with tamoxifen and phytoestrogens (genistein and daidzein). Anticancer Res. 23, 1119-1125.

Mylonas I., Makovitzky J., Richter D.U., Jeschke U., Briese V. and Friese K. (2003b). Immunohistochemical expression of the tumour marker CA-125 in normal, hyperplastic and malignant endometrial tissue. Anticancer Res. 23, 1075-1080.

Mylonas I., Jeschke U., Shabani N., Kuhn C., Balle A., Kriegel S., Kupka M.S. and Friese K. (2004a). Immunohistochemical analysis of estrogen receptor alpha, estrogen receptor beta and progesterone receptor in normal human endometrium. Acta Histochem. 106, 245-252.

Mylonas I., Jeschke U., Wiest I., Hoeing A., Vogl J., Shabani N., Kuhn C., Schulze S., Kupka M.S. and Friese K. (2004b). Inhibin/activin subunits alpha, beta-A and beta-B are differentially expressed in normal human endometrium throughout the menstrual cycle. Histochem. Cell Biol. 122, 461-471.

Mylonas I., Jeschke U., Shabani N., Kuhn C., Kriegel S., Kupka M.S. and Friese K. (2005). Normal and malignant human endometrium express immunohistochemically estrogen receptor alpha (ER-alpha), estrogen receptor beta (ER-beta) and progesterone receptor (PR). Anticancer Res. 25, 1679-1686.

Mylonas I., Makovitzky J., Hoeing A., Richter D.U., Vogl J., Schulze S., Jeschke U., Briese V. and Friese K. (2006). Inhibin/activin subunits

beta-A (-betaA) and beta-B (-betaB) are differentially localised in normal, hyperplastic and malignant human endometrial tissue. Acta Histochem. 108, 1-11.

Paech K., Webb P., Kuiper G.G., Nilsson S., Gustafsson J., Kushner P.J. and Scanlan T. S. (1997). Differential ligand activation of estrogen receptors Eralpha and ERbeta at AP1 sites. Science 277, 1508-1510

Remmele W. and Stegner H.E. (1987). Vorschlag zur einheitlichen Definierung eines immunreaktiven Score (IRS) für den immunhistochemischen Östrogenrezeptornachweis (ER-ICA) im Mammakarzinomgewebe. Pathologe 8, 138-140

Rey J.M., Pujol P., Dechaud H., Edouard E., Hedon B. and Maudelonde T. (1998). Expression of oestrogen receptor-alpha splicing variants and oestrogen receptor-beta in endometrium of infertile patients. Mol. Hum. Reprod. 4, 641-647.

Savouret J.F., Chauchereau A., Misrahi M., Lescop P., Mantel A., Bailly A. and Milgrom E. (1994a). The progesterone receptor. Biological effects of progestins and antiprogestins. Hum. Reprod. 9, 7-11.

Savouret J.F., Rauch M., Redeuilh G., Sar S., Chauchereau A., Woodruff K., Parker M.G. and Milgrom E. (1994b). Interplay between estrogens, progestins, retinoic acid and AP-1 on a single regulatory site in the progesterone receptor gene. J. Biol. Chem. 269, 28955-28962.

Snijders M.P., de Goeij A.F., Debets-Te Baerts M.J., Rousch M.J., Koudstaal J. and Bosman F.T. (1992). Immunocytochemical analysis of oestrogen receptors and progesterone receptors in the human uterus throughout the menstrual cycle and after the menopause. J. Reprod. Fertil. 94, 363-371

Taylor A.H., Guzail M., Wahab M., Thompson J.R. and Al-Azzawi F. (2005). Quantitative histomorphometric analysis of gonadal steroid receptor distribution in the normal human endometrium through the menstrual cycle. Histochem. Cell Biol. 123, 463-474

Vegeto E., Shahbaz M.M., Wen D.X., Goldman M.E., O'Malley B.W. and McDonnell D.P. (1993). Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. Mol. Endocrinol. 7, 1244-1255

Wang H., Critchley H.O., Kelly R.W., Shen D. and Baird D.T. (1998). Progesterone receptor subtype B is differentially regulated in human endometrial stroma. Mol. Hum. Reprod. 4, 407-412.

Accepted September 6, 2006