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Androgen, estrogen and progesterone receptor expression in the human uterus during the menstrual cycle☆

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Abstract

Cyclic changes in steroid receptor expression in endometrial cells are considered a reflection of its differential functions. Besides estrogen and progestogens, androgens have also been suggested to affect the biological function of the female reproductive tract. We investigated the distribution and intensity of immuno-cytochemical estrogen receptor (ER), progesterone receptor (PR) and androgen receptor (AR) staining in the various cell types of human endometrium and myometrium during the different menstrual cycle phases in 30 paraffin-embedded sections.

AR expression in endometrial stromal cells decreased gradually from early proliferative till mid secretory phase. In the late secretory phase, AR expression in all cell types distinguished. Staining of epithelial cells was moderate.

The disappearance of AR expression before cyclic separation of endometrial tissue may be causally related or just an epiphenomenon. Due to local competition for 5α-reduction of testosterone and the excess of progesterone in the secretory phase, the level of dihydrotestosterone (DHT) will be diminished. Hypothetically, if AR synthesis in endometrium would be DHT-dependent, it would explain the lack of AR expression in the late secretory phase.

Keywords: Androgen; Endometrium; Menstrual cycle; Steroid receptor

1. Introduction

The uterus is actively involved in providing the site of implantation. Expression of steroid receptors in endometrial glandular and stromal cells is considered a reflection of the differential functions with different requirements of hormone effects [1]. The effects of estrogen and progestogens on proliferation and differentiation of endometrial cells and their cyclic changes within the menstrual cycle have been studied extensively, but the significance of androgens has only recently been suggested [1–6].

Immuno-cytochemistry with the use of specific monoclonal antibodies allows the visualisation of estrogen (ER), progesterone (PR) and recently also androgen receptors (AR) in individual cell types of the human uterus. The purpose of this study was to re-examine the expression of estrogen and progesterone receptors and to examine the expression of androgen receptors in the different cell types of the human endometrium and in myometrium.

2. Material and methods

Thirty patients between 29 and 46 years of age who were scheduled for hysterectomy for benign gynaecological abnormalities were included in the study. They complained of menorrhagia ($n = 18$), dysmenorrhoea ($n = 8$) or uterine prolaps ($n = 4$). All women had a regular menstrual cycle; none of them had a history of exogenous hormonal therapy or recent disruption of the endometrial continuity (curettage, intra-uterine device) for at least 2 months preceding surgery.

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Immediately after surgery, the removed uteri were opened and full-thickness samples, including endometrium and underlying myometrium, were obtained of the midfundus. These specimens were fixed in buffered formaldehyde (3.7%) and routinely processed for paraffin embedding.

None of the uteri revealed histopathologic abnormalities. The endometrial dating criteria, described by Noyes et al. [7] were used to assess the phase of the menstrual cycle in haematoxylin–eosin stained sections of the midfundus region.

The histologic appearance of the endometrium was classified as M: menstruation (days 1–5), EP: early proliferative (days 6–9), LP: late proliferative (days 10–14), ES: early secretory (days 15–18), MS: mid secretory (days 19–22) and LS: late secretory (days 23–28) phase. For each of these phases specimens from five representative uteri were collected. Only specimens with a histologic appearance that agreed with the date of the last menstrual period were included.

The Medical Ethical Committee of the hospital approved the study. At the time of the sample collection no patient consent was required for studies of archival tissue in The Netherlands.

2.1. Immuno-staining

Steroid receptor immuno-cytochemistry was performed on paraffin-embedded sections. These sections were dewaxed, rehydrated and immersed for 10 min in methanol with 0.3% H_2O_2 to block endogenous peroxidase activity. The sections were immersed in 0.01 M sodium citrate buffer (pH 6.0) in a microwave-resistant container, and were then microwaved twice for 5 min at 700 W. After cooling to room temperature, the sections were rinsed in phosphate-buffered saline (PBS) (pH 7.6). All sections were then pre-incubated with 1% bovine serum albumin in PBS for 15 min. The hormone receptors were detected using anti-ER (1D5, Dako A/S, Glostrup, Denmark), anti-PR (mPRI, Transbio, Paris, France) and anti-AR (F39.4, Biogenex, San Ramon, USA) at a dilution of 1:150, 1:1000 and 1:100, respectively. The sections were incubated with the antibodies overnight at room temperature.

After rinsing in PBS, sections were incubated with goat-anti-mouse-biotin (Oncogene Science, Uniondale NY, USA) for 1 h at room temperature at a dilution of 1:400. After rinsing with PBS, the sections were incubated with strepABC (vector-elite-kit, Dako A/S, Glostrup, Denmark) for 1 h at room temperature at a dilution of 1:1:100 (ER:PR:AR).

As a negative control for background staining, the cells were stained using a mouse monoclonal antibody against BrdU (clone IIB5) [8]. As positive controls for ER and PR immuno-staining, we used ER and PR positive breast tumour specimens; for AR immuno-staining, we used human benign prostate tissue [9].

ER, PR and AR immuno-cytochemistry on paraffin sections has been validated in previous studies [9,10].

3. Methodology

Distribution and intensity of estrogen, progesterone and androgen receptor staining were scored semi-quantitatively in cavitary epithelial cells (A), functional laminal glandular epithelial cells (B), basal laminal glandular epithelial cells (C), functional laminal stromal cells (D), basal laminal stromal cells (E) and in myometrial cells (F). Classification of staining intensity was assessed on separate cohorts of 100 cells in three different high power fields (×400), thus, allowing assessment of heterogeneity in cell receptor content for a total number of 300 cells. Assessment of staining intensity profiles was random and blind: the menstrual cycle phase was unknown. After staining and steroid receptor scoring, the menstrual cycle phase was identified.

The intensity of specific staining was evaluated visually using a semi-quantitative analysis as described by McCarty et al. [11], as follows:

$$\text{Receptor score} = \sum_{i=0}^{i=4} P(i) \times i$$

where i is intensity of staining from 0 (no staining) to 4 (very intense staining) and $P(i)$ is percentage of cells stained with intensity i. The final receptor score was obtained by calculating the sum of the specific staining of the three cohorts. Intra-observer agreement was high ($r = 0.96, P < 0.00001$) and inter-observer agreement for the ER and PR scoring methods were also high ($r = 0.84$, $P < 0.0001$ and $r = 0.93$, $P < 0.00001$, respectively) [12]. In this study, we evaluated the intra-observer differences of classification of staining intensity of the sections immuno-stained for AR by re-scoring 21 of the sections.

3.1. Statistical analysis

Histological appearance combined with recorded last menstrual period, were stratified at all menstrual cycle phases and cell types A–F. These data were analysed to examine the relationships between menstrual cycle phase or uterine region and receptor scores, by using one way analysis of variance. For analysis of intra-observer differences, the Spearman's correlation coefficient was calculated.

A P-value of less than 0.05 was considered significant.

4. Results

Staining for ER, PR as well as for AR was found to be limited to nuclei in myometrial and endometrial cells. Receptor staining was not observed in connective tissue or vascular wall elements. Immuno-staining was not

observed in negative controls (receptor score (RS) 0) and could be confirmed in positive controls (RS 300 ± 0).

4.1. ER Immuno-staining

Basal laminal stromal cells (average of all phases± standard deviation = 120 ± 42), functional laminal stromal cells (107 ± 41) and myometrial cells (69 ± 41) showed less intensive staining of ER than epithelial cells. Receptor scores of glandular epithelial cells decreased from basal laminal endometrium (169 ± 70) via functional laminal endometrium (155 ± 58) to scores of cavitary epithelial cells (121 ± 61) (Table 1, Fig. 1).

Expression of ER reached a maximum in the early proliferative phase in all cell types of the uterus, with a minor (non-significant) decrease in the late proliferative and in the secretory phases.

In the early secretory phase, ER staining declined sharply in stromal and myometrial cells, whereas in epithelial cells ER staining persisted into the mid secretory phase. During the mid and late secretory phases, the ER content of smooth muscle cells and of pre-decidualizing stromal cells around spiral arterioles increased slightly.

Statistical significant differences between steroid receptor expression in the different menstrual cycle phases as well as in the different layers of the endometrium or myometrium, are shown by superscripts in Table 1.

4.2. PR Immuno-staining

PR expression of basal laminal glandular epithelial cells (average of all phases ± standard deviation = 184 ± 32) and functional laminal glandular epithelial cells (131± 103) was strong and reached a maximum in the late proliferative and early secretory phases, with a significant drop in the mid and late secretory phases. Nuclear PR staining in cavitary epithelial cells increased significantly from menstruation to the late proliferative and early secretory phase of the cycle; in the mid and late secretory phases PR decreased significantly. PR staining of basal laminal and functional laminal stromal cells increased gradually until the early and late proliferative phase, and diminished in the luteal phase of the menstrual cycle (Table 1, Fig. 1).

Myometrial cells showed consistently strong nuclear PR immuno-staining throughout the menstrual cycle (average of all phases ± standard deviation = 194 ± 12).

4.3. AR Immuno-staining

Evaluation of intra-observer correlation by repeated analysis of AR immuno-staining intensity showed significant agreement in receptor scores in glandular ($r = 0.89$, $P < 0.00001$), stromal ($r = 0.96$, $P < 0.00001$) and smooth muscle ($r = 0.93$, $P < 0.0001$) cells (Table 1, Figs. 1 and 2).

Table 1
Estrogen, progesterone and androgen receptor scores (± the standard deviations) for endometrial epithelial, glandular and stromal cells and for myometrial cells in the various phases of the menstrual cycle[a]

	M	EP	LP	ES	MS	LS
ER						
Cell type A	0 ag	216 ± 30 b	141 ± 43 ch	143 ± 30 ch	54 ± 13 d	53 ± 35 d
Cell type B	189 ± 41 c	244 ± 19 bf	191 ± 21 bf	237 ± 9 bf	93 ± 31 dh	78 ± 61 cdh
Cell type C	234 ± 25 bf	222 ± 10 bf	190 ± 36 c	223 ± 4 bf	88 ± 29 d	59 ± 37 a
Cell type D	153 ± 27 ch	160 ± 5 c	123 ± 9 h	76 ± 20 d	66 ± 6 d	63 ± 19 d
Cell type E	140 ± 28 c	174 ± 11 c	164 ± 92 bc	57 ± 22 d	96 ± 31 cd	92 ± 30 cd
Cell type F	91 ± 46 cdh	132 ± 38 ch	92 ± 27 ch	22 ± 5 g	60 ± 4 g	18 ± 21 g
PR						
Cell type A	41 ± 38 ag	109 ± 24 b	198 ± 4 c	225 ± 46 cdkm	21 ± 15 ag	23 ± 16 agh
Cell type B	88 ± 51 ab	178 ± 31 c	253 ± 4 e	250 ± 4 dm	15 ± 5 g	0 h
Cell type C	191 ± 40 c	240 ± 8 d	279 ± 5 f	243 ± 37 edkm	125 ± 42 bo	30 ± 13 ag
Cell type D	97 ± 6 b	107 ± 44 b	146 ± 11 o	136 ± 1c o	78 ± 15 b	47 ± 5 ag
Cell type E	108 ± 24 b	171 ± 38 co	102 ± 2 b	91 ± 3 b	108 ± 20 b	67 ± 25 ab
Cell type F	199 ± 22 c	193 ± 37 c	193 ± 35 c	188 ± 40 c	175 ± 27 c	214 ± 9 m
AR						
Cell type A	0 a	5 ± 8 a	14 ± 10 ab	2 ± 3 a	19 ± 15 ab	0 a
Cell type B	0 a	2 ± 3 a	0 a	0 a	5 ± 11 a	0 a
Cell type C	0 a	0 a	0 a	0 a	0 a	0 a
Cell type D	17 ± 2 b	71 ± 24 cf	62 ± 22 cd	37 ± 32 d	41 ± 22 d	0 a
Cell type E	30 ± 23 d	102 ± 28 e	79 ± 9 cf	38 ± 27 d	26 ± 12 d	0 a
Cell type F	70 ± 5 f	66 ± 9 cf	56 ± 16 cf	49 ± 20 dfg	33 ± 7 dg	0 a

[a] Corresponding letters mean non-significant differences; different letters mean significant differences. ER: estrogen receptor, PR: progesterone receptor, AR: androgen receptor, A: cavitary epithelial cells, B: functional laminal glandular epithelial cells, C: basal laminal glandular epithelial cells, D: functional laminal stromal cells, E: basal laminal stromal cells, F: myometrial cells, M: menstruation phase, EP: early proliferative phase, LP: late proliferative phase, ES: early secretory phase, MS: mid secretory phase, LS: late secretory phase.

In comparison with ER and PR receptor scores, immuno-staining of AR was less intense. Epithelial cells showed hardly any AR expression throughout the menstrual cycle.

In stromal cells, AR receptor scores reached a maximum in the early proliferative phase with a gradual decrease during the remaining cycle phases. This decrease was more obvious in basal laminal stromal cells than in functional laminal stromal cells.

In myometrial cells, there was no significant difference in immuno-staining in the menstruation, proliferative, early and mid secretory phases ($P = 0.938$). Myometrial cells in the late secretory phase did not stain for AR.

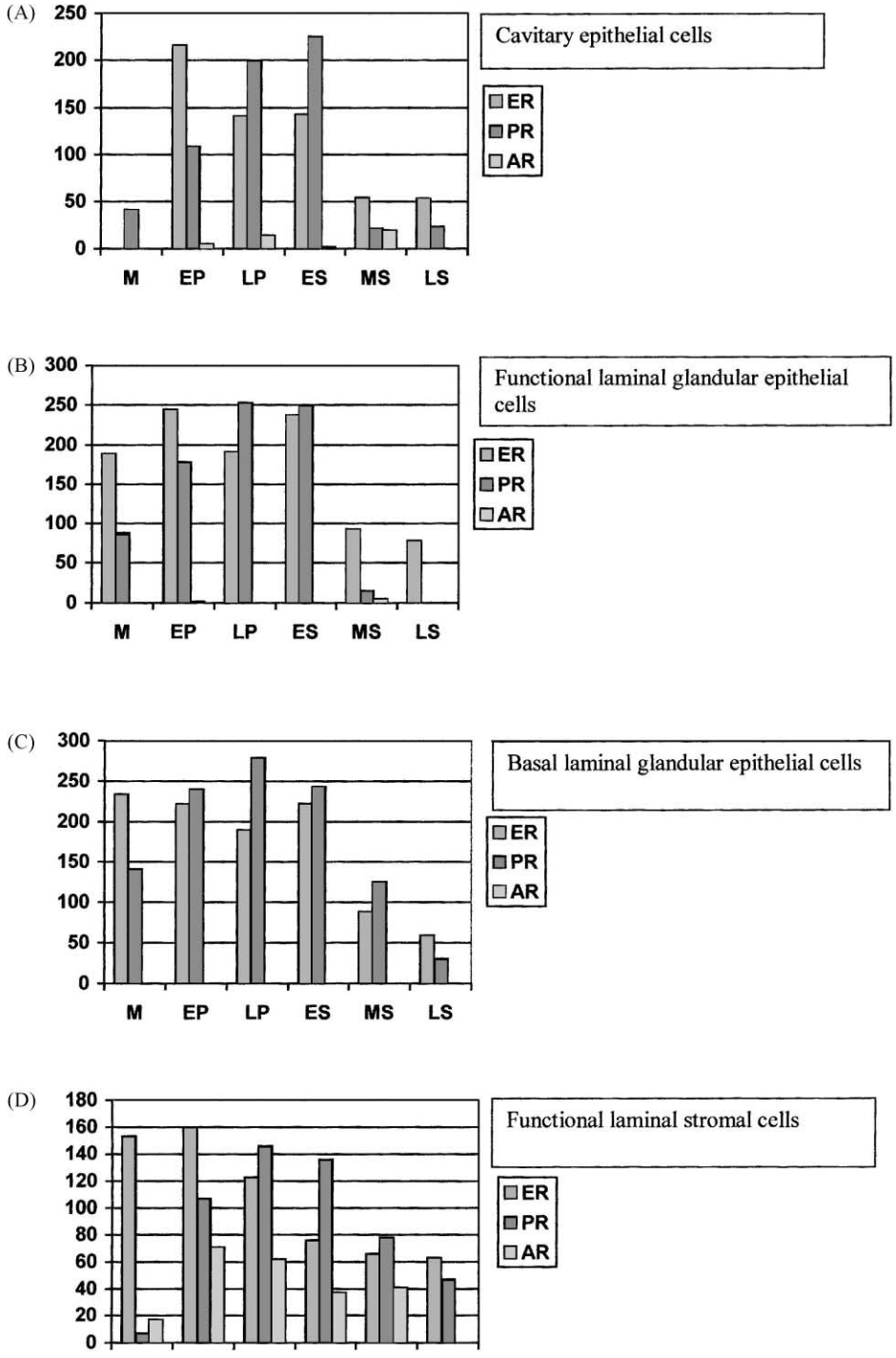

Fig. 1. Steroid receptor expression in different menstrual cycle phases. ER: estrogen receptor, PR: progesterone receptor, AR: androgen receptor, A: cavitary epithelial cells, B: functional laminal glandular epithelial cells, C: basal laminal glandular epithelial cells, D: functional laminal stromal cells, E: basal laminal stromal cells, F: myometrial cells, M: menstruation phase, EP: early proliferative phase, LP: late proliferative phase, ES: early secretory phase, MS: mid secretory phase, LS: late secretory phase.

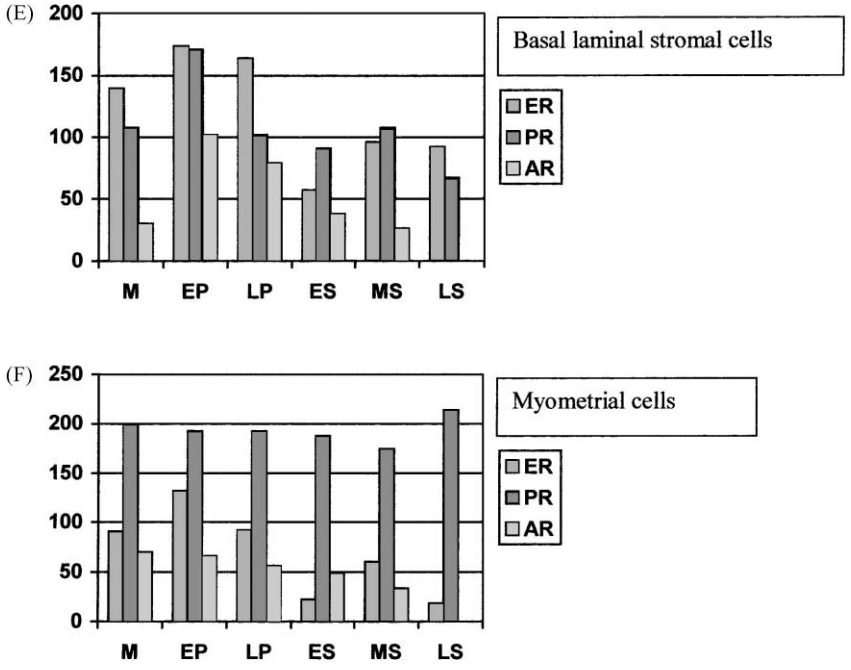

Fig. 1. (*Continued*).

AR immuno-staining of stromal and smooth muscle cells was more profound than AR staining of glandular cells, except for the late secretory phase, when no immuno-staining was found in any of the cell types.

5. Discussion

The present study describes the steroid receptor content of the various cell types of human endometrium and myometrium by immuno-staining of ER, PR and AR after microwave antigen retrieval pre-treatment of routinely fixed, paraffin-embedded tissue. Human endometrium and myometrium showed strong ER and PR expression in all uterine cell types during the proliferative phases of the menstrual cycle. In the secretory phases, ER and PR content decreased significantly. These results confirm the results of previous studies [2,12–15].

The knowledge of the AR content in the respective layers of the human endometrium and in the myometrium is incomplete. Investigating the presence of AR in human endometrium, Muechler et al. [3], Punnonen et al. [14] as well as Tamaya et al. [15] studied androgen binding to soluble proteins from the cytosol. Hirai et al. [4] studied the distribution of androgen receptor messenger RNA in rats by Northern blot analysis and in situ hybridisation using probes corresponding to the androgen-binding domain of the receptor. Also autoradiographic methods have been used for localising the AR [5,16,17].

Recently, localisation of AR in female reproductive tissues by immuno-histochemistry has been introduced [6,9,18,19]. By this method, androgen receptor expression in different normal human cell types during the phases of the menstrual cycle can be described in detail.

In this study, endometrial epithelial cells showed no AR expression during the menstrual cycle. Basal laminal stromal cells (Fig. 1) showed highest AR expression in the early proliferative phase with a gradually decrease during the late proliferative and early and mid secretory phases. In the mid secretory phase, AR staining was very low, in the late secretory phase, AR staining was not detected. AR immuno-staining of functional laminal stromal cells showed the same cyclic pattern, but was less intense than the staining in basal laminal stromal cells. Myometrial cells showed a gradual decrease of AR expression in the secretory phases. AR expression also disappeared in the late secretory phase.

Androgens have been shown to increase AR expression, possibly by a mechanism of auto-regulation. In rat prostatic tissue, Prins and Birch [20] showed immuno-detectable AR to decline within 2–7 days after castration-induced androgen withdrawal. AR immuno-staining returned within 1–3 days after testosterone replacement. A similar mechanism may be responsible for the pattern of AR expression during the cycle, if the uterine levels of dihydrotestosterone (DHT), the active metabolite of testosterone, may fluctuate. Such a conversion of testosterone to DHT is a prerequisite for androgen action in other genital tissues as well, e.g. during the development of the male external genitalia, urethra and prostate [21].

Presently, there is no evidence of large fluctuations of peripheral levels of testosterone or DHT during the menstrual cycle. However, post-ovulatory excess of progestogens may force these steroids as easily available substrates for 5α-reduction, resulting in high levels of 5α-reduced

progesterone in the secretory phases [21]. Due to local competition for 5α-reduction between testosterone (pmol serum concentrations) and progesterone (nmol serum concentrations) during the second half of the menstrual cycle, the reduction of progesterone may increase and reduction of testosterone may decrease in the late secretory phase of the cycle.

If AR synthesis in human endometrial cells, in analogy with that in prostate cells, would be DHT-dependent, the diminished level of DHT, in favour of the increased level of 5α-reduced progesterone, would explain the lack of AR immuno-staining in the late secretory phase of the menstrual cycle. The precipitous disappearance of progesterone from the circulation in the days preceding menses, and the

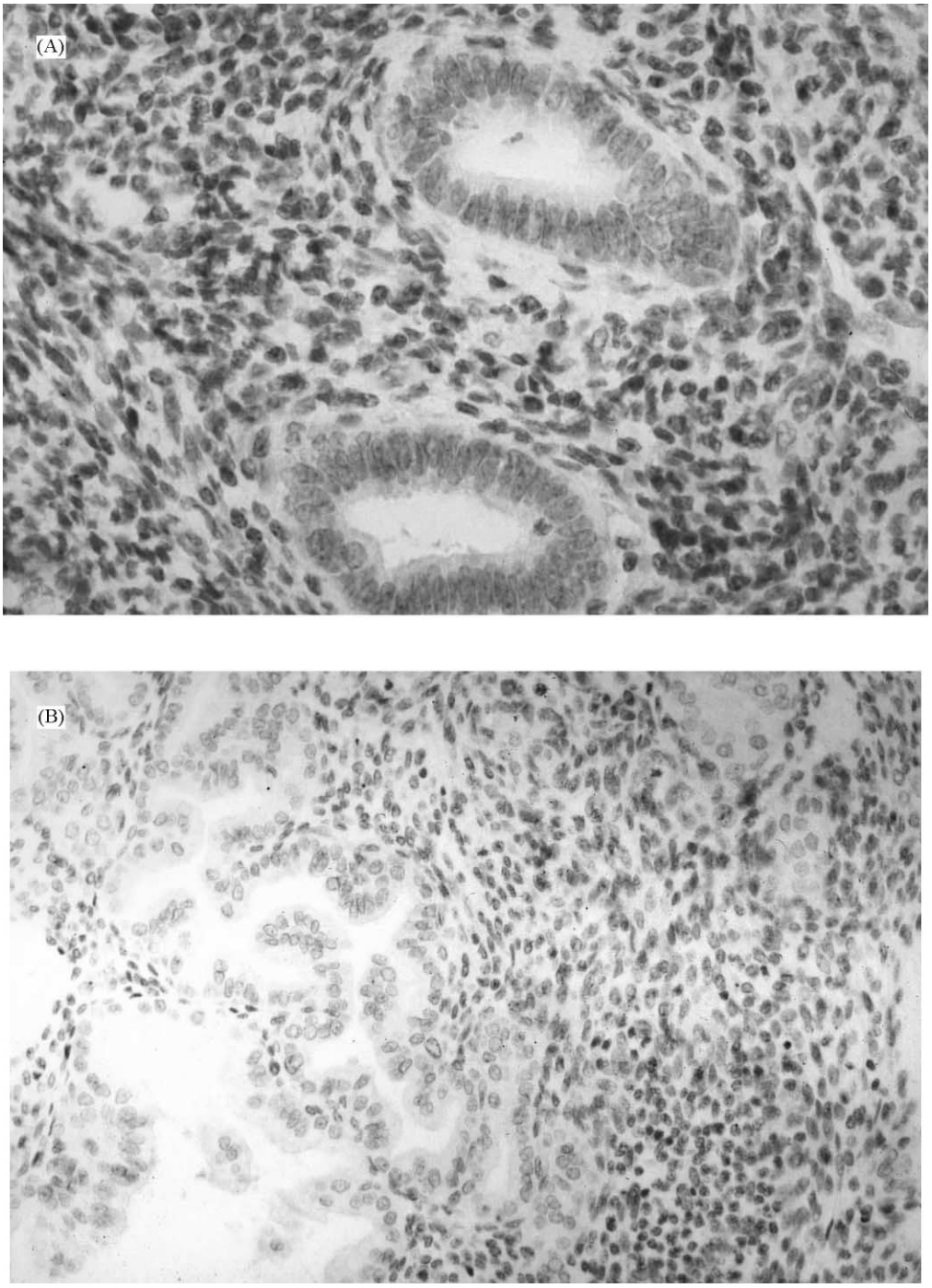

Fig. 2. (A) Staining for androgen receptor (AR) expression, shown by brown cells in the early secretory phase. Stromal cells show positive staining; all glandular cells show no staining for AR. (B) Staining for AR expression, shown by brown cells in the late secretory phase. Stromal cells as well as epithelial cells show no staining for AR. (C) Anti-BrdU mAb as a negative control for AR immuno-staining. No staining is shown.

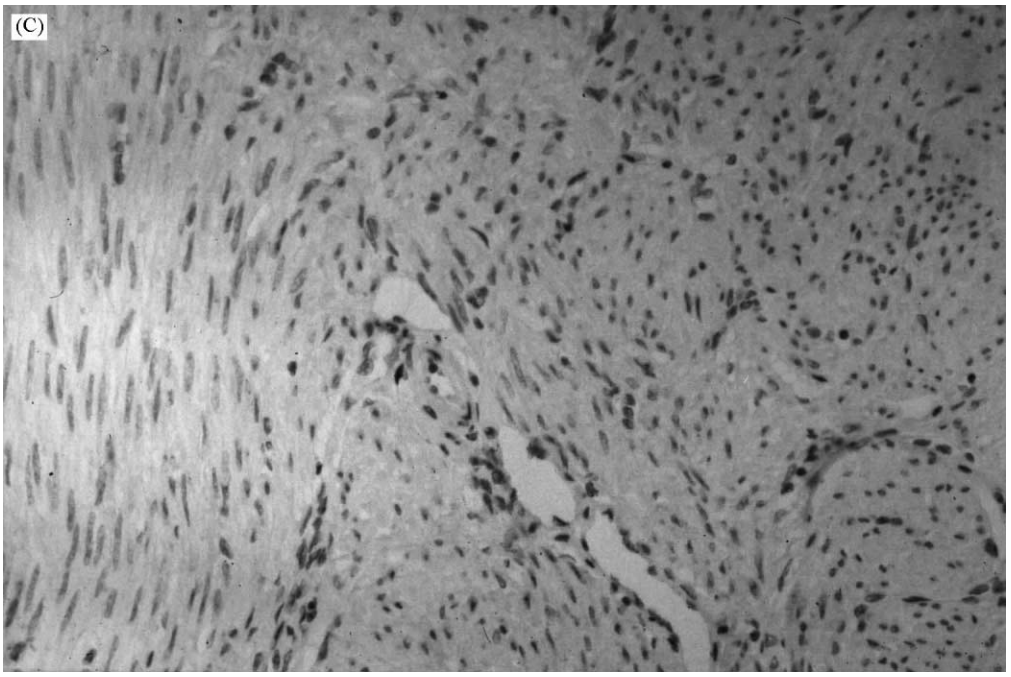

Fig. 2. (*Continued*).

resulting loss of competitive inhibition of testosterone conversion to DHT, would then explain the subsequent rapid return of AR in the early proliferative phase.

In the present study, ER, PR and AR expression have been visualised during the menstrual cycle. All three steroid receptors play definitively a role in function and morphology changes of human endometrial and myometrial cells throughout the menstrual cycle. The mode of androgen action and their modulative, or causal, role in the separation of endometrial tissue from its basal layer during the menstrual period has to be further investigated.

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References

[1] Noe M, Kunz G, Herbertz M, et al. The cyclic pattern of immunocytochemical expression of estrogen and progesterone receptors in human endometrium myometrial and endometrial layers: characterization of the endometrial–sub-endometrial unit. Hum Reprod 1999;14:190–7.

[2] Narukawa S, Kanzaki H, Inoue T, et al. Androgens induce prolactin production by human endometrial stromal cells in vitro. J Clin Endocrinol Metab 1994;78:165–8.

[3] Muechler EK. The androgen receptor of the human endometrium. Endocr Res 1987;13(1):69–84.

[4] Hirai M, Hirata S, Osada T, et al. Androgen receptor mRNA in the rat ovary and uterus. J Steroid Biochem Mol Biol 1994;49(1):1–7.

[5] Bonney RC, Scanlon MJ, Jones DL. Adrenal androgen concentrations in endometrium and plasma during the menstrual cycle. J Endocr 1994;101:181–8.

[6] Horie K, Takakura K, Imai K, et al. Immuno-histochemical localization of androgen receptor in the human endometrium, decidua, placenta and pathologic conditions of the endometrium. Hum Reprod 1992;7:1461–6.

[7] Noyes RW, Herig AT, Rock J. Dating the endometrial biopsy. Fertil Steril 1950;1:3–10.

[8] Schutte B, Reynders MMJ, Bosman FT, et al. Studies with anti-bromodeoxyuridine antibodies II: simultaneous detection of antigen and DNA synthesis by in vivo labeling of mouse intestinal mucosa. J Histochem Cytochem 1987;35:371–4.

[9] Janssen PJ, Brinkmann AO, Boersma WJ, et al. Immuno-histochemical detection of androgen receptor with monoclonal antibody F39.4 in routinely processed, paraffin-embedded human tissues after microwave pre-treatment. J Histochem Cytochem 1994;42:1169–75.

[10] Snijders M, Theunissen P, Debets-Te Baerts MJ, et al. Immunohistochemischer Nachweiss von Ostrogen- und Progesteronrezeptoren in Paraffinschnitten. Pathologe 1990;11:236–9.

[11] McCarty KS Jr, Milller LS, Cox EB, et al. Estrogeen receptor analyses: correlation of biochemical and immuno-chemical methods using monoclonal anti-receptor antibodies. Arch Pathol Lab Med 1985;109:716–21.

[12] Snijders MP, De Goeij AF, Debets-Te Baerts MJ, et al. Immunocytochemical analysis of estrogen and progesterone receptors in the normal human uterus throughout the menstrual cycle and after menopause. J Reprod Fert 1992;94:361–9.

[13] Ben-Hur H, Mor G, Insler V, et al. Assessment of estrogen receptor distribution in human endometrium by direct immuno-fluorence. Acta Obstet Gynecol Scand 1995;74:97–102.

[14] Punnonen R, Pettersson K, Vanharanta R. Androgen, estrogen and progestin cytosol receptor concentrations in the normal human endometrium. Effects of intrauterine device. Gynecol Obstet Invest 1994;17:73–7.

[15] Tamaya T, Murakami T, Okada H. Concentrations of steroid receptors in normal human endometrium in relation to the day of the menstrual cycle. Acta Obstet Gynecol Scand 1986;65:195–8.

[16] Takeda H, Mizuno T, Lasnitzki I. Autoradiographic studies of androgen-binding sites in the rat urogenital sinus and post-natal prostate. J Endocr 1985;104:87–92.
[17] Stumf WE, Sar M. Autoradiographic techniques for localizing steroid hormones. Meth Enzym 1975;36:135–56.
[18] Takeda H, Chodak G, Mutchnik S, et al. Immuno-histochemical localisation of androgen receptors with mono- and poly-clonal antibodies to androgen receptor. J Endocr 1990;126:17–25.
[19] Ruizeveld de Winter JA, Trapman J, Vermey M, et al. Androgen receptor expression in human tissues: an immuno-histochemical study. J Histochem Cytochem 1991;39:927–36.
[20] Prins GS, Birch L. Immuno-cytochemical analysis of androgen receptor along the ducts of the separate rat prostate lobes after androgen withdrawal and replacement. Endocrinology 1993;32:169–78.
[21] Wilson JD, Griffin JE, Russell DW. Steroid 5α-reductase 2 deficiency. Endocr Rev 1993;14:577–93.