

The cyclic pattern of the immunocytochemical expression of oestrogen and progesterone receptors in human myometrial and endometrial layers: characterization of the endometrial–subendometrial unit

M.Noë^{1,2}, G.Kunz¹, M.Herbertz^{1,2}, G.Mall² and G.Leyendecker^{1,3}

¹Departments of Obstetrics and Gynaecology and ²Pathology, Klinikum Darmstadt Academic Teaching Hospital to the University of Frankfurt, 64283 Darmstadt, Germany

³To whom correspondence should be addressed

Immunocytochemistry of oestrogen receptor (ER) and progesterone receptor (PR) expression of the whole uterine muscular wall and the endometrium was performed in order to obtain morphological and functional insights into the regulation of cyclic uterine peristalsis, which is confined to the endometrium and the subendometrial myometrium and serves functions such as rapid and sustained sperm transport. The study revealed that the subendometrial myometrium or stratum subvasculare with a predominantly circular arrangement of muscular fibres exhibits a cyclic pattern of ER and PR expression that parallels that of the endometrium, whereas the outer portion of the uterine wall composed of the stratum vasculare and supravasculare, which represents the bulk of the uterine musculature, does not exhibit a cyclic pattern of ER and PR expression. According to ontogenetic and phylogenetic data from the literature, the outer myometrium is of non-paramesonephric origin with functions confined to parturition, while the inner myometrial layer together with the glandular epithelium and the stroma of the endometrium is of paramesonephric origin with various functions during the cycle in addition to those during pregnancy and parturition. The inner quarter of the stratum vasculare adjacent to the stratum subvasculare constitutes a transitional zone in that the cyclicity of receptor staining becomes, in radial direction, gradually less expressed. Morphologically this zone corresponds to the inner part of the stratum vasculare where its muscular fibres blend with those of the stratum subvasculare.

Key words: endometrial–subendometrial unit/immunocytochemistry/oestradiol and progesterone receptors/sperm transport/uterine peristalsis

Introduction

Using immunocytochemical techniques, oestradiol receptors (ER) and progesterone receptors (PR) as well as the cyclic change of their expression have been demonstrated in human uterine tissues in several studies. A differential regulation of the receptor expression in the different uterine layers, such as the glandular epithelium and stroma of the endometrium

respectively, was considered a reflection of differential functions with differential requirements of hormone effects of these tissues during different phases of the cycle (Garcia *et al.*, 1988; Lessey *et al.*, 1988; Prentice *et al.*, 1992; Snijders *et al.*, 1992). In most of these studies only the myometrium immediately underlying the endometrium was analysed in this respect and considered as representative of the whole uterine muscular wall (Lessey *et al.*, 1988; Snijders *et al.*, 1992; Amsö *et al.*, 1994). It was not appreciated that the myometrium consists of different layers, which may display varying functions during different phases of the reproductive process. In fact, the human uterine muscular wall is composed of three layers, the stratum subvasculare adjacent to the endometrium with a predominantly circular arrangement of muscular fibres, the subserosal stratum supravasculare with a predominantly longitudinal arrangement of muscular fibres and, in between, the stratum vasculare, which consists of a three-dimensional mesh of short muscular bundles and constitutes the bulk of the uterine muscular wall in the adult female (Wetzstein, 1965).

The uterus is usually considered to be specialized, first, for the reception of the blastocyst by the endometrium and the continuous nourishment of the developing fetus and, second, for the eventual expulsion of the fetus. Furthermore, the uterine muscle is regarded to be normally functional for only a brief period following a lengthy gestation, unlike other smooth muscle organs (Garfield and Yallampalli, 1994; Romanini, 1994). Recently, it became evident, however, that the uterus, especially the non-pregnant one, is not a quiescent organ but is rather actively involved in the very early processes of reproduction, in addition to providing the site of implantation. It could be demonstrated that the non-pregnant uterus acts as a peristaltic pump during the menstrual cycle with directed sperm transport as one of the main functions (Kunz *et al.*, 1996; Leyendecker *et al.*, 1996). Vaginal sonography of uterine peristaltic activity (Birnholtz, 1984; De Vries *et al.*, 1990; Lyons *et al.*, 1991; Kunz *et al.*, 1996; Leyendecker *et al.*, 1996) has shown that the uterine peristaltic waves, under physiological conditions, only involve the stratum subvasculare of the myometrium, thus attributing a special and separate function to this inner layer of the myometrium. Furthermore, there is indirect (Lyons *et al.*, 1991; Kunz *et al.*, 1996; Leyendecker *et al.*, 1996) and direct evidence (Kunz *et al.*, 1998a) that the uterine peristaltic activity is under the control of the ovarian dominant structure.

In view of these data it appeared to be necessary to re-examine the expression of the oestradiol and progesterone receptors in the different endometrial and myometrial layers during the menstrual cycle and in post-menopausal women.

The data obtained were related to morphological, ontogenetic as well as phylogenetic data from the literature.

Materials and methods

Uterine tissue samples

Non-pregnant uteri were obtained from 27 normally cycling women, aged 29–49 years and three post-menopausal women undergoing hysterectomy for various reasons but not for myometrial and endometrial pathology. All pre-menopausal women had regular cycles and no history of hormone therapy for at least 6 months. The post-menopausal women had not received hormone replacement therapy. In the pre-menopausal women, the respective phases of the cycle were ensured by correlating the dates of the last menstrual period with histological findings according to the usual histological dating method (Dallenbach-Hellweg and Poulsen, 1984). In selecting the uteri for this study, special care was taken that the specimens were devoid of endometrial and myometrial pathology. Microscopically, all the sections used showed a normal endometrial and myometrial structure.

After resection, hysterectomy specimens were fixed with 4% buffered (phosphate-buffered saline, pH7.2; Merck, Darmstadt, Germany) formaldehyde for 24 h. At least two (left and right side) transmural uterine samples were taken from the mid-region of the uterine corpus. In some cases additional samples of the lower parts of the corpus were taken. From each of these samples 4–20 frontal sections were obtained that contained the whole uterine wall from the endometrium to the serosa or from the endocervix to the parametrium.

Immunostaining

Oestrogen and progesterone receptor immunocytochemistry was performed on paraffin-embedded sections (thickness 3 µm). After mounting, deparaffination and rehydration, unmasking of antigen was carried out using high-temperature techniques (Shi *et al.*, 1991). The sections were then incubated with blocking serum (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA, USA) for 30 min at 37°C. This was followed by overnight incubation at room temperature with primary anti-oestrogen-receptor mouse monoclonal antibody (Novocastra Laboratories, Newcastle upon Tyne, UK), developed against prokaryotic recombinant protein corresponding to the full-length oestrogen receptor molecule, at a dilution of 1:40 in Tris buffer (Siquia-Aldrich Chemicals, Deisenhofen, Germany) and primary anti-progesterone-receptor mouse monoclonal antibody (Novocastra Laboratories), developed against synthetic peptide corresponding to a site of predicted high antigenicity on the human progesterone receptor and binding to both known types of nuclear progesterone receptors (PR-A and PR-B; Viville *et al.*, 1997), dilution 1:40 in Tris buffer respectively. The sections were then incubated at 37°C with biotinylated secondary anti-mouse antibody followed by avidin biotin peroxidase complex (Vectastain Elite ABC, Vector Laboratories), each for 30 min in the first and 15 min in the second run and were washed with buffer after each step. The slides were flooded with freshly prepared diaminobenzidine–imidazole–hydrogen peroxide solution (Merck) for 45 min in the dark. After washing with water the sections were counterstained with haematoxylin, washed with water again, dehydrated (with ethylene alcohol and xylene; Merck) and mounted. Evaluation of this method was carried out by comparison of a series of 40 slides from the same specimen, which were treated under different unmasking conditions. Microwave heating at 600 W for 7 min was used, which gave the best morphological and staining results with the least cellular destruction. In order to obtain optimal staining differentiation, various concentrations of the primary antibodies were tested, with a dilution of 1:40 giving the best results.

Sections of receptor-positive mammary carcinomas served as positive controls; negative controls were made using sections of receptor-positive mammary carcinomas without incubation with primary antibody and several oestrogen-receptor-negative tissues (e.g. from tonsils and non-gynaecological carcinomas), which were prepared according to the standard protocol of oestrogen and progesterone receptor immunohistochemistry.

Evaluation

Results of specific staining were evaluated using a semiquantitative method. The staining intensity was graded as 0 = no, 1 = weak, 2 = moderate and 3 = strong staining respectively. The number of cells/area and the degree of positivity respectively for the endometrial glandular epithelium and stroma, as well as three myometrial regions, were compared. The myometrium of the stratum supravasculare (subserosal myometrium), of the stratum vasculare and of the stratum subvasculare (subendometrial myometrium) were counted out separately with high-power fields. The subserosal and the subendometrial endometrium were counted out completely. In the broad stratum vasculare at least 20–25 high-power fields, which were evenly distributed along the transmural axis between the two other layers, were selected and counted out. The tissue was examined by a single observer and confirmed by a second observer. The immunoreactive score (IRS) was calculated using the following equation:

$IRS = SPi (i + 1)$, where $i = 1, 2$ or 3 , and Pi is the percentage of stained cells for each intensity, according to the method described by Lessey *et al.* (1988).

The immunoreactive score of positively stained cells per uterine region was determined by taking the arithmetic mean of the values of all counted high-power fields. There was a high reproducibility of the method in that, in a test series, the inter-assay variation over the full range of IRS never exceeded 10%.

The data obtained from individual uterine specimens of the pre-menopausal women were grouped according to phase of the menstrual cycle, with days 1–6 representing the early proliferative ($n = 5$), days 7–9 representing mid-proliferative ($n = 6$), days 10–15 representing the peri-ovulatory ($n = 5$), days 16–21 representing the early to mid-secretory ($n = 5$) and with days 22–28 representing the mid- to late secretory phase ($n = 6$) of the cycle respectively.

Statistical analysis

Statistical analysis was performed with Student's *t*-test. Significance was assumed when $P < 0.05$.

Results

Oestrogen receptors (ER) during the cycle

The results are summarized in Figure 1, while Figure 3 shows representative sections of the different uterine layers throughout the menstrual cycle. The uterine layers, i.e. the glandular epithelium, the stroma, the subendometrial myometrium, the stratum vasculare and the subserosal myometrium respectively, behave differently with respect to the cyclic pattern of the immunoreactive scores. There was a dramatic cyclic change of the IRS of the glandular epithelium and stroma of the endometrium. The immunostaining of the subendometrial myometrium exhibited a cyclic pattern that nearly completely paralleled that of the endometrium. The IRS of the inner portion of the stratum vasculare, which consists of roughly one-third of the whole stratum vasculare, exhibited a reduced cyclic pattern in comparison with that of the subendometrial

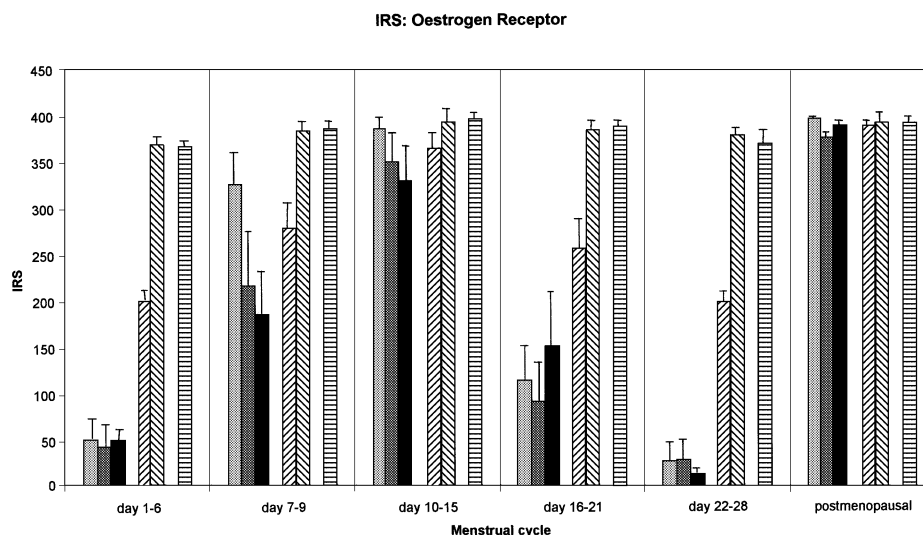


Figure 1. Immunoreactive scores (IRS) of oestrogen receptors of the epithelial (▨) and stromal parts of the endometrium (■), of the stratum subvasculare (str. subvasc. ■), the inner (str. vasc. I ▨) and outer (str. vasc. II ▩) parts of the stratum vasculare as well as of the stratum supravasculare (str. suprasc. ▨) of the myometrium in different phases of the menstrual cycle as well as in post-menopausal women (mean \pm SE).

myometrium, while those of the outer two-thirds of the stratum vasculare showed, as the stratum supravasculare, no cyclic pattern at all. Thus, with respect to ER immunostaining, the bulk of the uterine muscular wall exhibits no cyclic changes, with a high IRS throughout the whole cycle. Cyclic changes of IRS are confined to the endometrium, the subendometrial myometrium and, to a lesser extent, to the inner portion of the stratum vasculare. The cyclic changes of IRS of ER of the stromal and epithelial component of the endometrium as well as of the subendometrial endometrium (early proliferative and late secretory versus late proliferative phase respectively) were statistically significant ($P < 0.001$) as was the difference of IRS of ER between the stratum subvasculare and the stratum supravasculare and outer portion of the stratum vasculare respectively (early proliferative phase and late secretory phase) ($P < 0.001$), whereas differences of the subserosal myometrium and the outer portion of the stratum vasculare during the menstrual cycle were not significant.

Progesterone receptor (PR) during the cycle

The results are summarized in Figure 2, while representative sections of the different uterine layers throughout the menstrual cycle are demonstrated in Figure 4. Qualitatively, the cyclic pattern of immunoreactivity for PR is similar to the respective pattern of ER immunoreactivity. The bulk of the myometrium consisting of the stratum supravasculare and the larger outer portion of the stratum vasculare again does not exhibit a cyclic pattern of PR immunoreactivity at all. Quantitatively, the other layers show a variable degree of cyclic changes of immunostaining with the glandular epithelium showing the most marked ones and the inner third of the stratum vasculare exhibiting the least marked changes. The cyclic changes of IRS of PR of the stromal and epithelial component of the endometrium and the stratum subvasculare (early proliferative and late secretory versus peri-ovulatory phase respectively)

were statistically significant ($P < 0.01$), whereas the outer portions of the uterine muscular wall showed no statistically significant differences ($P > 0.1$).

The subendometrial myometrium behaving like endometrium with respect to ER expression comprised about one quarter of the total thickness of the muscular wall (as estimated in sections with a completely negative internal layer during the early proliferative and late secretory phases respectively). The boundary between the outer, positively stained portion of the wall and the inner negative layer was not sharp. Rather, a transitional zone (TZ) existed within the stratum vasculare, which comprised roughly another quarter of the total muscular wall. Thus, a continuous increase of positive staining was found when moving within the transitional zone of the stratum vasculare towards the outer myometrium.

Two specimens of the lower uterine portion were studied during the early follicular phase and two during the late secretory phase respectively, in addition to those specimens of the mid-region of the same uteri. There was no difference in staining characteristics of the layers along the longitudinal axis of the uterus.

Post-menopausal patients

There was maximal immunocytochemical expression in all layers for oestradiol and progesterone receptors.

Discussion

Immunocytochemistry with the use of specific monoclonal antibodies allows the visualization of oestradiol and progesterone receptors in individual cells and tissue layers of the uterus as a target organ of ovarian steroids. With respect to the distribution of oestradiol and progesterone receptors in the glandular and stromal part of the endometrium and its cyclical change throughout the menstrual cycle, this study in general

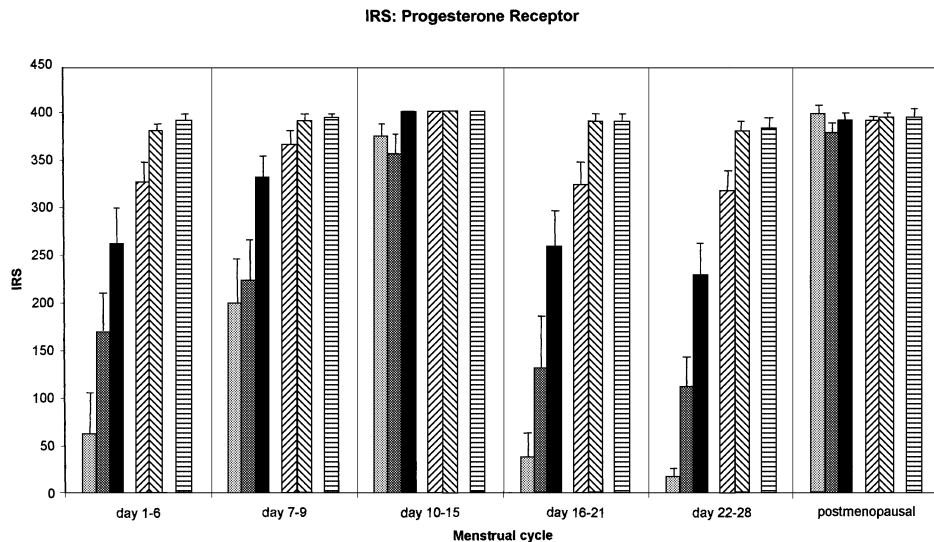


Figure 2. Immunoreactive scores (IRS) of progesterone receptors of the glandular and stromal parts of the endometrium, of the stratum subvasculare, the inner and outer parts of the stratum vasculare as well as of the stratum supravasculare of the myometrium in different phases of the menstrual cycle as well as in post-menopausal women (mean \pm SE). For abbreviations and key, see Figure 1.

confirms previous results (Garcia *et al.*, 1988; Lessey *et al.*, 1988; Snijders *et al.*, 1992; Amso *et al.*, 1994; Shiozawa *et al.*, 1996). No gradient along the longitudinal uterine axis could be observed in myometrium and endometrium, also confirming previous results (Snijders *et al.*, 1992; Richards and Tiltman, 1995).

The novel finding of this study, however, is the demonstration that there is no consistent pattern of steroid-receptor staining across the myometrial wall. While the expression of steroid receptors of the subendometrial myometrium paralleled the cyclic pattern of the endometrial epithelium and stroma, the outer part of the myometrium consisting of the stratum supravasculare and most of the stratum vasculare showed no cyclic pattern at all but rather strong staining throughout the whole cycle.

The high expression of both ER and PR in the uterine layers of post-menopausal women suggests that the expression of ER and PR is constitutive for uterine tissue and that the cyclic change of the receptor expression is, in addition to up-regulation by oestradiol, primarily a matter of down-regulation by changing progesterone concentrations (Kraus and Katzenellenbogen, 1993; Iwai *et al.*, 1995; Graham and Clarke, 1997). The functional significance of cell-specific differential receptor regulation may be viewed in a suppression of hormone action in the respective tissue in the presence of high circulating concentrations of this hormone required for continuing action in another tissue (King *et al.*, 1980; McCormack and Glasser, 1980; Lessey *et al.*, 1988; Shiozawa *et al.*, 1996).

In addition to the observation that cyclically changing uterine peristaltic activity is confined to the subendometrial myometrium (Lyons *et al.*, 1991; Kunz *et al.*, 1996), the cyclically changing ER and PR pattern suggests strongly that the subendometrial myometrium is functionally distinct from the rest of the myometrium and is rather part of a functional unit of which the other components are the endometrial epithelium and the endometrial stroma. This conjecture is

supported by both data from comparative morphology and human embryology.

Phylogenetic data of the uterine muscular wall show that, in all vertebrates listed (Table I), the muscular wall of the uterus or of the uterine part of the oviduct respectively is composed of a stratum subvasculare with a circular arrangement of the muscular fibres. While in birds (Van Tienhoven, 1961) and monotremata (Van den Broek, 1933) the stratum vasculare constitutes the only muscular layer of the uterus/oviduct, in marsupials (Lierse, 1965) and rodents (Garfield and Yallampalli, 1994) the outer stratum supravasculare with predominantly longitudinal muscular fibres has been acquired additionally. In the human, the loose mesenchymal mesh of the stratum vasculare, as observed in the rodent, has developed into a inner third muscular layer consisting of a three-dimensional mesh of irregular short muscular bundles and constituting the bulk of the uterine muscular wall (Werth and Grusdew, 1898; Wetzstein, 1965).

The acquisition of muscular layers in addition to the stratum subvasculare appears to be related to the forces that are required for parturition under the condition of viviparity and especially under the condition of the human with the relative disproportion between the fetal head and the pelvis. This view is not contradicted but rather supported by the finding of a stratum supravasculare in the tortoise *Gopherus polyphemus*. In this species, the outer layer evolved to provide forces in a longitudinal direction required for simultaneous oviposition of the eggs that are ovulated as a clutch (Palmer and Guilette, 1988). In sequential ovulators, such as birds and also in the opossum, the single circular layer is apparently sufficient for the transport of the egg in the oviduct and for functioning as a sphincter (Van den Broek, 1933; Van Tienhoven, 1961).

The acquisition of uterine muscular layers in addition to the circular stratum subvasculare late in phylogeny corresponds to the late development of these layers during human embryology (Table II). The stratum vasculare and supravasculare respect-

Table I. Phylogenetic and functional aspects with respect to oviposition and viviparity of the oviductal/uterine muscular layers. Stratum subvasculare: the inner layer with a predominantly circular arrangement of muscular fibres. Stratum vasculare: the middle layer with short muscular bundles extending in virtually all directions. Stratum supravasculare: the outer layer with a predominantly longitudinal arrangement of muscular fibres

Species			Oviductal or uterine muscular layers	Function of the oviductal/myometrial force	References
Reptiles (tortoises)	Stratum subvasculare	–	Stratum supravasculare	Simultaneous oviposition	Palmer and Guillette (1988)
Birds	Stratum subvasculare	–	–	Sequential oviposition	Van Tienhoven (1961)
Monotremata	Stratum subvasculare	–	–	Oviposition	Van den Broek (1933)
Marsupials	Stratum subvasculare	–	Stratum supravasculare	Viviparity	Lierse (1965)
Rodents	Stratum subvasculare	–	Stratum supravasculare	Viviparity	Garfield and Yallampalli (1994)
Human	Stratum subvasculare	Stratum vasculare	Stratum supravasculare	Viviparity	Werth and Grusdew (1898) Wetzstein (1965)

Table II. The ontogeny of the layers of the human uterine wall with respect to embryonic and fetal age

Weeks of pregnancy (post-ovulatory)	Features	References
5+6	Paramesonephric ducts develop	Müller (1829), Faulconer (1951)
7+3	Paramesonephric ducts are separated widely	O'Rahilly (1989)
8	Paramesonephric ducts have fused (primordial uterus)	Koff (1933), Matejka (1959)
13–18	Mesenchyme with circular arrangement around uterus and tubes	Werth and Grusdew (1898), Hunter (1930)
22–26	Circular layers of the primordial myometrium (stratum subvasculare)	Werth and Grusdew (1898)
27–39 (and postnatally)	Stratum vasculare and supravasculare	Werth and Grusdew (1898)

ively only develop in the third trimester of gestation or even postnatally (Werth and Grusdew, 1898), whereas the anlage of the stratum subvasculare is already apparent at the beginning of the second trimester with a circular arrangement of mesenchymal cells around the primordial uterus and tubes. The ontogenetically early formation of the inner circular layer is also documented by a kind of fundo-cornual raphe of the stratum subvasculare (Werth and Grusdew, 1898) (Figure 5) that results from the fusion of the two paramesonephric ducts and their mesenchymal elements to form the primordial uterus. The fundo-cornual raphe appears to be an important structural detail with respect to uterine physiology. The bipartition of the circular subendometrial myometrium in the upper part of the uterine corpus and its separate continuation through the cornua into the respective tubes is apparently the morphological basis for directed sperm transport into the tube ipsilateral to the dominant follicle (Kunz *et al.*, 1996, 1998b). Furthermore, the fundo-cornual raphe of the subendometrial myometrium might be a site of constant microtrauma due to uterine peristalsis and hyperperistalsis, from where endometriosis and adenomyosis may arise (Leyendecker *et al.*, 1996, 1998). The cervical part of the uterus is morphogenetically strongly influenced by vaginal elements in that the inner paramesonephric component, the glandular epithelium and the circular muscular layer, is sheathed and blended with vaginal musculature and connective tissue (Werth and Grusdew, 1898) (Figure 5).

The immunocytochemical findings of this study reflect astoundingly these phylogenetic and ontogenetic data. In the uterus, a cyclic expression of ER and PR can only be demonstrated in those layers that are derived from the paramesonephric ducts, i.e. the glandular epithelium and the stroma of the endometrium and the stratum subvasculare of the myometrium, whereas the expression of the ER and PR of the muscular tissue of non-Müllerian origin, the stratum supravasculare and the outer portion of the stratum vasculare (Werth and Grusdew, 1898), does not display a cyclic pattern. The intermediate behaviour of the inner quart of the stratum vasculare in this respect corresponds with the morphological data of Werth and Grusdew (1898) in that muscular elements of paramesonephric origin blend with those of non-paramesonephric origin.

Thus, the human uterus is composed, ontogenetically and phylogenetically, of two separate organs, the paramesonephric endometrial–subendometrial unit and the outer non-paramesonephric myometrium (Figure 5). Both the stratum vasculare and supravasculare subserve parturition. This requires growth and myometrial quiescence in a lengthy gestation (Soloff, 1989), the constant action of both oestradiol and progesterone and, hence, the constant expression of their respective receptors (Katzenellenbogen *et al.*, 1979; Graham and Clarke, 1997).

The functions of the endometrial–subendometrial unit are more complex. They consist in the cyclic preparation of the endometrium for implantation and in the cyclically changing uterine peristalsis with the main function of uterine sperm transport (De Vries *et al.*, 1990; Kunz *et al.*, 1996; Leyendecker *et al.*, 1996). Inflammatory defence may also be regarded as a specific and phylogenetically old function of the endometrial–subendometrial unit (Leiva *et al.*, 1994; Surveyor *et al.*, 1995; Xu *et al.*, 1995; Loke and King, 1996; Gipson *et al.*, 1997) since, in lower animals, the Müllerian ducts end in a cloaca and, in the human, the cervical mucus does not act as a barrier

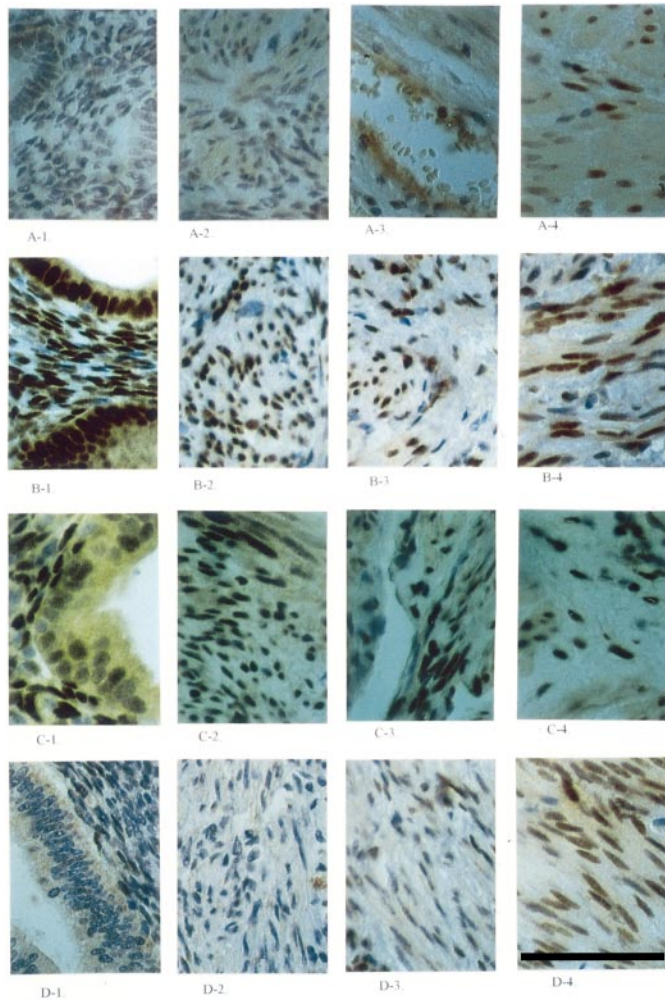


Figure 3. Representative sections of immunostaining for oestrogen receptors of the uterine wall throughout the menstrual cycle. The sections depict the endometrium (1), the stratum subvasculare (2), the stratum vasculare (3) and the stratum supravasculare (4) respectively, during the early proliferative (A), peri-ovulatory (B), early secretory (C) and late secretory (D) phases respectively of the menstrual cycle. Bar = 50 μ m.

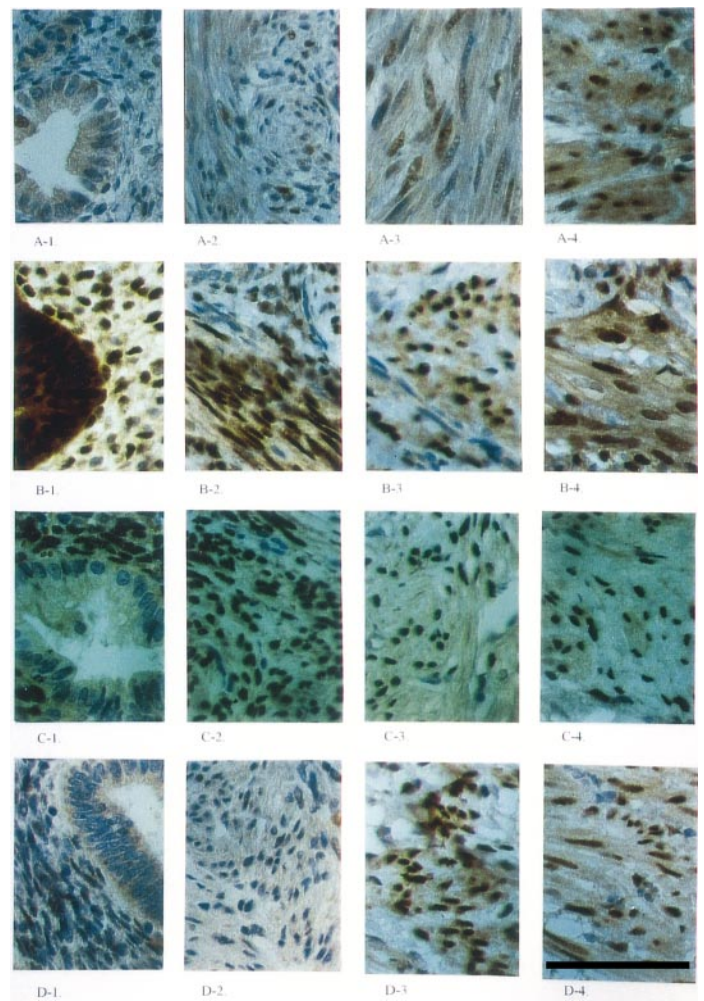


Figure 4. Representative sections of immunostaining for progesterone receptors of the uterine wall throughout the menstrual cycle. The sections depict the endometrium (1), the stratum subvasculare (2), the stratum vasculare (3) and the stratum supravasculare (4) respectively, during the early proliferative (A), peri-ovulatory (B), early secretory (C) and late secretory (D) phases respectively of the menstrual cycle. Bar = 50 μ m.

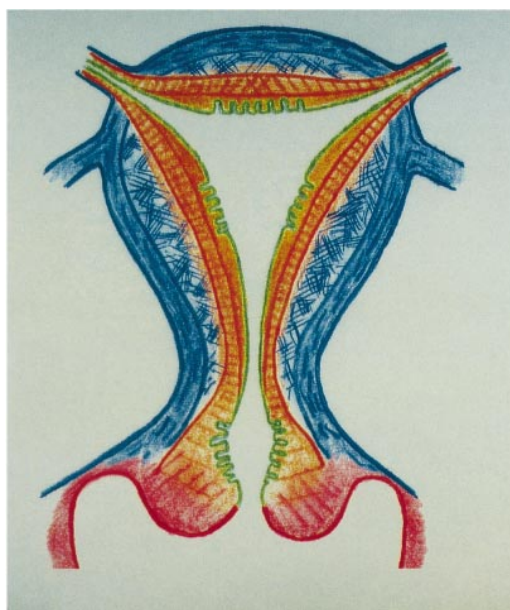


Figure 5. A schematic representation of the endometrial-subendometrial unit ('archimetra') within the human uterus based on the immunocytochemical results of this study and the morphological and ontogenetic data of Wetzstein (1965) and Werth and Grusdew (1898) respectively. The endometrial-subendometrial unit is composed of the glandular (green), the stromal part of the endometrium and the stratum subvasculare of the myometrium with predominantly circular muscular fibres (orange). Ontogenetically, the endometrial-subendometrial unit is derived from the paramesonephric ducts (green) and their surrounding mesenchyme (orange). The bulk of the human myometrium does not originate from the paramesonephric ducts (blue). It consists of the stratum vasculare with a three-dimensional meshwork of short muscular bundles and the stratum supravasculare with predominantly longitudinal muscular fibres. The stratum vasculare is the phylogenetically most recent acquisition and, in contrast to the endometrial-subendometrial unit, both the stratum vasculare and supravasculare develop late during ontogeny. The stratum vasculare and supravasculare surround the uterine corpus and extend caudally only to the uterine isthmus. There is a transitory zone within the stratum vasculare adjacent to the stratum subvasculare where muscular fibres of the two layers blend (orange margin of the stratum vasculare). The endocervical mucosa is the most caudal structure derived from the paramesonephric ducts. The underlying circular muscular fibres, which are progressively diminishing in caudal direction, and the accompanying connective tissue blend with vaginal tissue elements (red) to form the vaginal portion of the cervix.

for passive ascension of inert particles and sperm in neither phase of the cycle (unpublished) (Faundes *et al.*, 1981; Kunz *et al.*, 1996). The cyclic changes in ER and PR expression in the endometrial–subendometrial unit apparently meet the requirements of a cyclic ovarian control over these functions.

In conclusion, immunocytochemistry of the whole uterine muscular wall and the endometrium revealed that the subendometrial myometrium exhibits a cyclic pattern of ER and PR expression that parallels that of the endometrium, whereas the outer portion of the uterine wall does not exhibit a cyclic pattern of ER and PR expression. The data were correlated with morphological, embryological and phylogenetic data of the myometrial wall, and it became evident that both endometrium and subendometrial myometrium form a functional unit with various cyclic reproductive functions in addition to those during gestation and parturition. This endometrial–subendometrial functional unit is phylogenetically an ancient organ within the uterus and could be termed the ‘archimetra’ with reference to Werth and Grudew (1898), who used the term archimyometrium to describe the ontogenetically old character of the stratum subvasculare of the myometrium.

References

- Amso, N.N., Crow, J. and Shaw, R.W. (1994) Comparative immunohistochemical study of oestrogen and progesterone receptors in the Fallopian tube and uterus at different stages of the menstrual cycle and the menopause. *Hum. Reprod.*, **9**, 1027–1037.
- Birnholz, J. (1984) Ultrasonic visualization of endometrial movements. *Fertil. Steril.*, **41**, 157–158.
- Dallenbach-Hellweg, G. and Poulsen, H. (1984) *Atlas der Histopathologie des Endometriums*. Springer Verlag, Berlin, Heidelberg, New York, Tokyo.
- De Vries, K., Lyons, E.A., Ballard, G. *et al.* (1990) Contractions of the inner third of the myometrium. *Am. J. Obstet. Gynecol.*, **162**, 679–682.
- Faulconer, R.J. (1951) Observation on the origin of the Müllerian groove in human embryos. *Contrib. Embryol. Carnegie Inst.*, **34**, 159–164.
- Faundes, A., Montes de Oca, V., Brache, V. and Alvarez-Sanchez, F. (1981) Sperm migration at different stages of the menstrual cycle. *Int. J. Gynaecol. Obstet.*, **19**, 361–366.
- Garcia, E., Bouchard, P., de Brux, J. *et al.* (1988) Use of immunocytochemistry of progesterone and oestrogen receptors for endometrial dating. *J. Clin. Endocrinol. Metab.*, **67**, 80–87.
- Garfield, R.E. and Yallampalli, C. (1994) Structure and function of uterine muscle. In Chard, T. and Grudzinskas, J.G. (eds), *Cambridge Reviews in Human Reproduction 4. The Uterus*. pp. 54–93.
- Gipson, I.K., Ho, S.B., Spur-Michaud, S.J. *et al.* (1997) Mucin genes expressed by human female reproductive tract epithelia. *Biol. Reprod.*, **56**, 999–1011.
- Graham, J.D. and Clarke, C.L. (1997) Physiological action of progesterone in target tissues. *Endocrinol. Rev.*, **18**, 502–519.
- Hunter, R.H. (1930) Observations on the development of the female genital tract. *Contrib. Embryol. Carnegie Inst.*, **22**, 91–108.
- Iwai, M., Kanzaki, H., Fujimoto, M. *et al.* (1995) Regulation of sex steroid receptor gene expression by progesterone and testosterone in cultured human endometrial stromal cells. *J. Clin. Endocrinol. Metab.*, **80**, 450–454.
- Katzenellenbogen, B.S., Bhakoo, H.S., Ferguson, E.R. *et al.* (1979) Estrogen and antiestrogen action in reproductive tissues and tumors. *Recent Progr. Hormone Res.*, **35**, 292–300.
- King, R.J.B., Townsend, P.T., Whitehead, M.I. *et al.* (1980) Biochemical analyses of separated epithelium and stroma from endometria of premenopausal and postmenopausal women receiving estrogen and progestins. *J. Steroid Biochem.*, **14**, 979–987.
- Koff, A.K. (1933) Development of the vagina in the human female genital tract. *Contrib. Embryol. Carnegie Inst.*, **24**, 59–90.
- Kraus, W.L. and Katzenellenbogen, B.S. (1993) Regulation of progesterone receptor gene expression and growth in the rat uterus: modulation of estrogen actions by progesterone and sex steroid hormone antagonists. *Endocrinology*, **132**, 2371–2379.
- Kunz, G., Beil, D., Deininger, H. *et al.* (1996) The dynamics of rapid sperm transport through the female genital tract. Evidence from vaginal sonography of uterine peristalsis (VSUP) and hysterosalpingoscintigraphy (HSSG). *Hum. Reprod.*, **11**, 627–632.
- Kunz, G., Noë, M., Herberitz, M. and Leyendecker, G. (1998a) Uterine peristalsis during the follicular phase of the menstrual cycle. Effects of oestrogen, antioestrogen and oxytocin. *Hum. Reprod.*, *Update*, **0**, 00.
- Kunz, G., Herberitz, M., Noë, M. and Leyendecker, G. (1998b) Sonographic evidence of a direct impact of the ovarian dominant structure on uterine function during the menstrual cycle. *Hum. Reprod.*, *Update*, **0**, 00.
- Leiva, M.C., Hasty, L.A. and Lyttle, C.R. (1994) Inflammatory changes of the endometrium in patients with minimal-to-moderate endometriosis. *Fertil. Steril.*, **62**, 967–972.
- Lessey, B.A., Killiam, A.S.P., Metzger, D.A. *et al.* (1988) Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. *J. Clin. Endocrinol. Metab.*, **67**, 334–340.
- Leyendecker, G., Kunz, G., Wildt, L. *et al.* (1996) Uterine hyperperistalsis and dysperistalsis as dysfunctions of the mechanism of rapid sperm transport in patients with endometriosis and infertility. *Hum. Reprod.*, **11**, 1542–1551.
- Leyendecker, G., Kunz, G., Noë, M. *et al.* (1998) Endometriosis: a dysfunction and disease of the archimetra. *Hum. Reprod.*, *Update*, **4**, 751–762.
- Liersé, W. (1965) The wall construction and blood vessel system of the uterus of the opossum. *Acta Anatomica*, **60**, 152–263.
- Loke, Y.W. and King, A. (1996) Immunology of human implantation: an evolutionary perspective. *Hum. Reprod.*, **11**, 283–286.
- Lyons, E.A., Taylor, P.J., Theng, X.H. *et al.* (1991) Characterization of subendometrial myometrial contractions throughout the menstrual cycle in normal fertile women. *Fertil. Steril.*, **55**, 771–774.
- Matejka, M. (1959) Die Morphogenese der menschlichen Vagina und ihre Gesetzmäßigkeiten. *Anat. Anz.*, **106**, 20–37.
- McCormack, S.A. and Glasser, S.R. (1980) Differential response of individual uterine cell types from immature rats treated with estradiol. *Endocrinology*, **106**, 1634–1649.
- Müller, J. (1829) Über Wolfschen Körper bei den Embryonen der Frösche und Kröten. *Meckels Archiv für Physiologie*, 65–70.
- O’Rahilly, R. (1989) Prenatal human development. In Wynn, R.M., Jollie W.P. (eds), *Biology of the Uterus*. Second edition. Plenum Medical Book Co., pp. 35–55.
- Palmer, B.D. and Guilette, L.J. (1988) Histology and functional morphology of the female reproductive tract of the tortoise *Gopherus polyphemus*. *Am. J. Anat.*, **183**, 200–211.
- Prentice, A., Randall, B.J., Weddell, A. *et al.* (1992) Ovarian steroid receptor expression in endometriosis and in two potential parent epithelia: endometrium and peritoneal mesothelium. *Hum. Reprod.*, **7**, 1318–1352.
- Richards, P.A. and Tiltman, A.J. (1995) Anatomical variation of the oestrogen receptor in normal myometrium. *Virchows Arch.*, **427**, 303–307.
- Romanini, C. (1994) Measurement of uterine contractions. In Chard, T. and Grudzinskas, J.G. (eds), *Cambridge Reviews in Human Reproduction 4. The Uterus*. pp. 337–355.
- Shi, S.-R., Key, M.E. and Kalra, K.L. (1991) Antigen retrieval in formalin-fixed paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J. Histochem. Cytochem.*, **39**, 741–748.
- Shiozawa, T., Li, S.-F., Nakayama, K. *et al.* (1996) Relationship between the expression of cyclins/cyclin-dependent kinases and sex-steroid receptors/Ki67 in normal human endometrial glands and stroma during the menstrual cycle. *Mol. Hum. Reprod.*, **2**, 745–742.
- Snijders, M.P.M.L., de Goeij, A.F.P.M., Debets-Te Baerts, M.J.C. *et al.* (1992) Immunohistochemical analysis of oestrogen receptors and progesterone receptors in the human uterus throughout the menstrual cycle and after the menopause. *J. Reprod. Fert.*, **94**, 363–371.
- Soloff, M.S. (1989) Endocrine control of parturition. In Wynn, R.M. and Jollie, W.P. (eds), *Biology of the Uterus*. Plenum Medical Book Company, pp. 559–607.
- Surveyor, G.A., Gendler, S.J., Pemberton, L. *et al.* (1995) Expression and steroid hormonal control of MUC-1 in the mouse uterus. *Endocrinology*, **136**, 3639–3647.
- Van den Broek, A.J.P. (1933) Gonaden und Ausführungsgänge. In Bolk, L., Göppert, E., Kallius, E. and Lubosch, W. (eds), *Handbuch der vergleichenden Anatomie der Wirbeltiere*. Urban und Schwarzenberg, Berlin, pp. 1–154.
- Van Tienhoven, A. (1961) Endocrinology of reproduction in birds. In Young, W.C. (ed.), *Sex and Internal Secretion*. Volume 2. Williams and Wilkins, Baltimore, USA. pp. 1088–1196.

- Viville, B., Charnock-Jones, D.S., Sharkey, A.M. *et al.* (1997) Distribution of the A and B forms of the progesterone receptor messenger ribonucleic acid and protein in uterine leiomyomata and adjacent endometrium. *Hum. Reprod.*, **12**, 815–822.
- Wetzstein, R. (1965) Der Uterusmuskel: Morphologie. *Arch. Gynecol.*, **202**, 1–13.
- Werth, R. and Grudew, W. (1898) Untersuchungen über die Entwicklung und Morphologie der menschlichen Uterusmuskulatur. *Arch. Gynäkol.*, **55**, 325–409.
- Xu, L., Wu, Q. and Wang, B. (1995) Antibacterial components of rat uterus. *Hua Hsi I Ko Ta Hsueh Hsueh Pao*, **26**, 261–265 (medline research).

Received on June 8, 1998; accepted on October 1, 1998