NUCLEAR CHANNEL SYSTEM IN SECRETORY ENDOMETRIUM IN NATURAL CYCLE AND AFTER ESTRADIOL-PROGESTERONE SUBSTITUTION IN TRANSMISSION ELECTRON MICROSCOPY

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Abstract

The objective of the study was to ascertain if the estradiol-progesterone substitution used for the preparation of endometrium which has been shown to elicit supraphysiological levels of circulating progesterone may also induce demonstrable changes in the ultrastructural morphology of human secretory endometrium which is characterised by three progesteron-dependent structures, the so called postovulatory triad: nuclear or nucleolar channel system, giant mitochondrion and subnuclear glycogen.

The study was approved by the institutional Ethic Committee and included 24 patients of one IVF centre who signed informed consent. Two sequentional endometrial biopsy specimens were taken on the 5th and 7th day after ovulation in one natural cycle and on the 5th and 7th day after the initiation of progesterone addition in another substituted cycles in the same patient. The surface and glandular epithelial cells were examined under transmission electron microscope (TEM).

The appearance of nuclear channel system (NCS), giant mitochondria and glycogen was the same in both natural and substituted cycles, without abnormalities and deformations. Cells with NCS were found, mostly in clusters on discute parts, in 55 to 70 % of ultrathin sections. The relative number of NCS positive specimens was not statistically different between the cycles or between the day +5 and the day +7 within different type of cycle. Some NCS positivity was detected more often (in 79 % and 66 %, resp.) in substituted cycles than in spontaneous ones. The percentage of NCS positive cells was also higher (median 17 % and 12 %, resp.) in substituted cycles but the differences were not statistically significant.

With some caution regarding the inherent limited reproducibility of the TEM findings we can conclude that the oral hormonal substitution used in our setting does not markedly alter endometrial ultrastructural morphology.

Key words: human endometrium, ultrastructure, nuclear channel system, oral estradiol-progesterone substitution

INTRODUCTION

Endometrium undergoes cyclic growth and development with the sole purpose of successful establishment of pregnancy. Uterine endometrial cells are regulated directly by ovarian steroids and indirectly by various growth factors and cytokines. The two days’ interval of maximal uterine receptivity which usually occurs around cycle days 20 to 24 is called nidation or implantation window (1). This period is characterised by expression of many different endometrial products (2). The receptivity of endometrium is a result of a coordinated hormonal regulation by ovarian steroids. The estrogen and progesterone receptors in the epithelial cells as well as in the stromal cells mediate the effects of these steroids. Their expression is therefore one of the crucial factors in this regulation (3,4).

Ultrastructure of surface and glandular epithelial cells of secretory endometrium is characterized by three main progesterone - dependent structures: nuclear channel system (NCS), giant mitochondria and subnuclear glycogen deposits which are sometimes called the postovulatory triad (5).

The most unique of these, nuclear channel system (NCS) is a peculiar ultrastructure, which was so far found only in the epithelial cells of the human secretory endometrium. The structure

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was first found in a close relation to nucleolus and therefore was called „Kanälchen und Maschenstruktur im Nukleolus“ (6), basket-like nucleolar structure (7), or nucleolar channel system (8). Now it is known to be situated in the karyoplasm without relation to the nucleolus and therefore denoted as the nuclear channel system. Last year Issac (9) called NCS “intranuclear endoplasmatic reticulum”.

Detailed three-dimensional structure and developmental stages (formation and regression) of NCS were studied in serial sections (10, 11) and by high voltage electron microscopy (12, 13).

NCS is a spherical structure, about 1 µm in diameter. On the periphery it is formed by several rows of channels (tubules) with the diameter of 60 - 100 nm, which are arranged in spirals round an electron lucent centre with a finely granular material. The tubules are formed by invagination of the inner membrane of the nuclear envelope (14) and thus their lumina communicate with the perinuclear space. The formation of NCS is dependent on the increased level of progesterone, as was also demonstrated in vitro (15).

Giant mitochondria is uncommonly large organelle with the diameter of about 5 µm. They are often found closed to a single cistern of endoplasmatic reticulum. Their inner surface is often covered with ribosomal granules and the outer surface is smooth (16).

Small amount of glycogen was detected in endometrial cells before ovulation (17), but the highest concentrations are seen during secretory phase during which they progressively increase (18, 19).

METHODS

The study group included 24 infertile women that had cryopreserved embryos from a previous IVF cycle in a single infertility center over the three-year interval 2001-2003. All women gave their written consent to the study, which was approved by the Institutional Review Board of the Faculty of Medicine, Palacký University, Olomouc.

Women had a history of infertility of more than 12 months, were less than 40 years old, had regular menstrual cycle, normal basal serum levels of gonadotrophins (FSH<10 IU/l) and prolactin. Infertility evaluation revealed endometriosis, tubal, idiopathic or male factor infertility. Patients were examined in the course of one spontaneous and one hormonally substituted cycle.

1. **Spontaneous cycle:** All subjects monitored urinary LH excretion by themselves daily from the cycle day 10 using commercial kit (Simtech Biorex Inc, USA). From the cycle day 11, repeated vaginal ultrasound examination (Hewlett Packard, vaginal probe 7.5 MHz) and serum LH, estradiol (E2) and progesterone (P) determination were performed every second day till the ovulation (day 0). Endometrial biopsies were performed on the luteal days +5 and +7 after ovulation (O+5, O+7).

2. **Substituted cycle:** Hormonal substitution protocol according to the Jones Institute (20) was started on the first day of another cycle using progressively increasing dose of estradiol valerate (2 mg/d from day 1 to 6, then 4 mg/d from day 7 to 10 and 6 mg/d from day 11 to 15). From day 11 serum levels of E2 and P were measured as in the spontaneous cycle. If on day 15 the endometrial thickness reached 8 mm or more, 600 mg/d of micronised P was started and the dose of estradiol valerate was decreased to 4 mg/d. Endometrial biopsies were taken on days +5 and +7 after P addition (P+5, P+7).

Sequential uterine endometrial biopsies were taken from the opposite sides of the anterior wall and fundus using the Novak curette while patient was under sedation. The tissue was immediately placed into fixation solution.

Specimens for TEM were prepared as previously described (11). Up to about 10 embedded pieces of tissue were cut into semithin sections and stained with toluidine blue for examining in the light microscope. From the selected surface epithelium area the targeted ultrathin sections were cut. Ultrathin sections of glandular epithelium were prepared only from unsuitably orientated pieces of tissue in which surface epithelium could not be obtained. Samples were exam-
ined under the TEM microscope Zeiss Opton 109, in 3 000x-10 000x magnification, by a single operator. Since the endometrium shows variable morphology from one area to the other, the amount of NCS in all developmental stages (e.g. developing, fully developed and regressing, see (11)) was counted in all epithelial cells of each ultrathin section (mostly between 50 to 200 cells in total) to increase the likelihood of a representative observation. The counting of the NCS was done directly on the fluorescent screen of the TEM. The percentual proportion of nuclei containing the NCS to all the nuclei in the area of the ultrathin section was calculated.

Statistical analysis was performed by $\chi^2$ test. Probability level of $p<0.05$ was considered statistically significant.

RESULTS

In all patients the number of NSC in the endometrial surface epithelial cells and glandular epithelial cells were counted together. During both cycles, spontaneous and after oral estrogen-progesterone substitution, ultastructure of NCS was normal, without abnormalities and deformations. One typical fully developed NCS from a substituted cycle is shown in Fig. 1.

Usually a nucleus contains only one, exceptionally two NCS. During early development, full development and early regression, NCS as an intranuclear structure exists in contact with cytoplasm (Fig 2, spontaneous cycle) and nuclear pores are preserved (Fig 2a, detail).

The comparison of the frequency of NCS positive biopsy specimens in TEM in all endometrial epithelial cells detected on the day +5 and the day +7 of the spontaneous and estrogen-progesterone substituted cycles in the same 24 patients is shown in Table I.

**Fig 1** Fully developed nuclear channel system situated near the karyoplasm. Invagination of cytoplasm enables communication of NCS electron lucent center with cytoplasmatic space. TEM, patient No 18, substituted cycle, O+7, magnification 40 000x.
Fig 2 Fully developed NCS in contact with nucleolus. TEM, patient No 18, natural cycle, O+7, magnification 24 000x.

Fig 2A Detail from Fig.2 shows a cross section of some NCS tubules, well preserved nuclear porus and a perinuclear cistern with outer and inner nuclear membrane. TEM, patient No 18, natural cycle, O+7, magnification 100 000x.
In some cases (up to 16 %) the suitable ultrathin section could not be obtained from any pieces of bioptic specimen. For statistical evaluation $\chi^2$ test with adjustments for missing values was used. The differences between the cycles or between the day +5 and the day +7 in either type of cycle were not statistically significant.

The NCS positivity at least in one of the sequential biopsies was detected more often in substituted cycles than in spontaneous cycles (in 79 % and 66 %, resp.) Cells with NCS were found in clusters on discrete parts of ultrathin sections. Therefore, it is not surprising that large variations in the percentage of NCS positive cells were observed in individual patients and samples. The percentage of NCS positive cells in endometrial epithelium ranged from 3 to 60 % (median 12 %) in spontaneous cycles and from 1 to 45 % (median 17 %) in substituted cycles. In addition, the other two components of the triad, giant mitochondria and glycogen were in substituted cycles morphologically normal.

**DISCUSSION**

NCS, giant mitochondria and subnuclear glycogen are specific ultrastructures that appear in the human endometrial epithelium in the postovulatory period. Their formation is initiated under the influence of all progestational steroids with acyl group in 17-$\beta$ position of the D-ring (21, 22).

A possible role for the NCS in implantation may be derived from the fact that the emergence of NCS coincides with the time of blastocyst attachment to the endometrium. Some authors (9) speculate that the blastocyst selects clusters of epithelial cells containing NCS to ensure proper attachment. They found such clusters in only about 5% of all the endometrial epithelial cells in the secretory endometrium of healthy women.

Estrogen-progestin substitution used for some assisted reproduction procedures may alter the delicate balance of endogenous ovarian hormones. In a previous study (23) we have shown that such treatment significantly increases serum P and sex hormone - binding globulin (SHBG) levels, sometimes even into the supraphysiological range. A moderate but significant increase of the expression of progesterone receptors was also observed (24).

As far as the NCS are concerned we have not observed any significant changes in the occurrence and morphological ultrastructural features of NCS between the endometrial epithelial cells from spontaneous and substituted cycles. With regard to the inherent high variability of the TEM results we can conclude with due caution that the oral substitution used in our setting does not negatively alter endometrial receptivity.

Favourable results of 20 cryoembryotransfers already performed in patients included in our study (25) also seem to support this presumption.
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