Ultrastructural and Histochemical Markers of Endometrial Secretion Induction in Habitual Miscarriage

N. A. Ilizarova, I. O. Marinkin*, T. A. Ageeva*, N. P. Bgatova*, V. M. Kuleshov*, and S. V. Aidagulova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 148, No. 10, pp. 468-472, October, 2009
Original article submitted July 27, 2009

Biphasic hormone therapy at the stage of pregestation treatment of patients with habitual miscarriages stimulates the expression of progesterone receptors in the endometrium during the secretory phase of the menstrual cycle with full-value ultrastructural rearrangement of the endometrial glandular components in comparison with the patients receiving metabolic therapy alone.

Key Words: *habitual miscarriage; endometrium; electron microscopy; immunohistochemistry*

Habitual miscarriage (HM) is one of the most complex medical social problems [2-4]. Progesterone deficiency plays an important role in infertility and HM [6,9]. One of the most sensitive target tissues in hormone therapy is the endometrium, which is very rapidly remodeled under the effect of sex hormones [5].

The presence of estrogen receptors in endometrial cells is the key factor providing the transformation of hormone pulses into structural changes [1]. Estrogens provide endometrial proliferation processes, simultaneously inducing the synthesis of progesterone receptors. Insufficient expression of progesterone receptors, resultant from inadequate content of estrogens, underlies the deficiency of the lutein phase and is one of the HM mechanisms. A discrepancy between the stromal changes and the glandular status is often seen in the majority of endometrial biopsy specimens collected during stimulated ovulation. Among the signs of early secretion are glandular subnuclear vacuoles, whose morphogenesis and role remain an object of discussions [1,7].

We carried out a comparative immunohistochemical analysis of progesterone receptor expression and studied the ultrastructural modifications of the endometrium in HM in the course of cyclic metabolic and hormone therapies.

Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk; *Novosibirsk State Medical University, Federal Agency for Health Care and Social Development, Russia. ***Address for correspondence:*** pathol@soramn.ru. N. A. Ilizarova

MATERIALS AND METHODS

Endometrial biopsy specimens from 80 women with HM (groups 1, 2, 3) receiving pregestation therapy according to 3 protocols during 3 menstrual cycles and 20 biopsy specimens from women with a history of gestation with good outcomes (group 4; control) were analyzed.

Group 1 (n=20) women received cyclic metabolic therapy 3 times daily: vitamin E (100 mg), calcium pantothenate (0.1 g), and lipoic acid (0.012 g) on days 5-15 of the cycle and vitamin E (100 mg), folic acid (1 mg), and potassium orotate (0.5 g) on days 16-25. Group 2 (n=30) received cyclic metabolic therapy in combination with duphaston (dydrogesterone), a natural analog of progesterone, in a daily dose of 20 mg on days 16-25. Group 3 (n=30) received a combination of cyclic metabolic and biphasic hormone therapies, including femostone (1 mg 17β-estradiol and 10 mg didrogesterone) on days 1-28 and duphaston (didrogesterone) in a daily dose of 10 mg on days 16-25.

The groups were matched for age (group 1: 27.30±1.03; group 2: 27.40±1.01; group 3: 27.60±1.02; and group 4: 26.20±0.95 years). All women participating in

0007-4888/09/1484-0663

the study gave their written consent to comprehensive examinations and therapy of HM. Functional diagnosis, measurements of blood levels of gonadotropins (follicle stimulating (FSH) and luteinizing hormones (LH)), estradiol (days 3-5 of the cycle), and progesterone (days 22-24) were carried out before therapy and during cycle 3 of preparation to gestation.

Biopsy specimens of the endometrium collected on days 22-24 of the cycle before and after therapy were fixed in 4% paraformaldehyde. Paraffin (stained with hematoxylin and eosin in combination with Pearles' reaction), semithin (stained with azur II), and ultrathin sections (contrasted with uranyl acetate and lead citrate) were examined. The expression of progesterone receptors in the endometrial epithelium and stroma was studied by the immunohistochemical method (two-step streptavidine-biotine method) with Dako reagents on paraffin sections. Antibodies PjR 636 to progesterone receptors served as primary antibodies. The reaction products were visualized with 3,3-diaminobenzidene tetrahydrochloride, the sections were poststained with hematoxylin, and the densities of glandular and endometrial stromal labeled cells were evaluated. Sections of tumor tissue with known expression of progesterone receptors (breast cancer) served as the positive control; for negative control, the primary antibodies were replaced with nonimmune serum.

Light microscopy was carried out using a universal Leica DM 4000B microscope with a Leica DFC 320 digital camera, ultrastructural studies were carried out in a JEM 1010 electron microscope at accelerating voltage of 80 kV.

RESULTS

Endometrial atrophy, including glandular and glandular epithelial atrophy with a significant reduction in the number of intranuclear vacuoles, was observed during the middle secretion phase of the cycle in HM patients (groups 1-3 before therapy). Glandular polymorphism by the degree of maturation was worthy of note; just solitary glands corresponded to the stage of secretion phase. Solitary nodes of spiral arteries were seen (2-3 transverse sections in a visual field). Immunohistochemical study of the endometrium showed poor expression of progesterone receptors: 12% glandular cells and 10% stromal cells in the endometrium were positively stained (Fig. 1, *a*).

In contrast to HM, the endometrial structure of controls on day 23 of the cycle corresponded to mature middle stage of the secretion phase, the percentage of progesterone-positive glandular and stromal cells being 60 and 57%, respectively (Fig. 1, *b*).

In group 1 (cyclic metabolic therapy), the index of labeled cells in biopsy specimens was 13% for the endometrial glandular epithelium and 11% for stromal cells. Presumably, the level of corpus luteum hormones remained low in metabolic therapy and/or the sensitivity of glandular and stromal cell receptors to progesterone was low.

Combination of metabolic therapy with duphaston (group 2) induced the expression of progesterone receptors in 55% glandular epitheliocytes and 29% stromal cells (Fig. 1, *c*). This was paralleled by structural and functional changes in the endometrium, more even and corresponding to the periods of the menstrual cycle. Glands of stellate and pilus-like shape with wide lumens and plicated contours predominated; they were lined with low prismatic epithelium and filled with secretion. The decidual reaction of the stroma, including formation of nodes of spiral arteries, was more pronounced than in group 1.

In group 3 (combination of metabolic and biphasic hormone therapy during 3 cycles), the expression of progesterone receptors in the endometrium reached the level of the control group: 58% in glandular epithelial cells and 55% in the stroma (Fig. 1, *d*), which was paralleled by positive restructuring.

TABLE 1. Blood Concentrations of Gonadotropins and Sex Hormones in HM (*M*±*m*)

Parameter	Group 1		Group 2		Group 3		Group 4 (control)
	before therapy	after 3 months	before therapy	after 3 months	before therapy	after 3 months	
FSH, U/liter	4.8±0.5	4.6±0.4	4.9±0.5	4.7±0.4	4.50±0.39	3.6±0.2	3.4±0.2
LH, U/liter	6.0±0.8	5.9±0.5	5.8±0.6	5.7±0.4	6.1±0.7	5.8±0.5	5.7±0.3
Estradiol, ng/ml	189.0±20.1*	200.0±22.1*	191.0±22.0*	205.0±22.2*	185.0±19.1*	251.0±23.2^{+}	261.0±26.1
Progesterone, mg/liter	9.7±0.9*	9.8±0.8*	12.1±1.1*	47.4±3.3^{+}	10.4±0.7	59.1±4.1^{+}	60.6±3.1

Note. $p<0.05$ compared to: *group 4 (control) and $^{+}$during therapy.

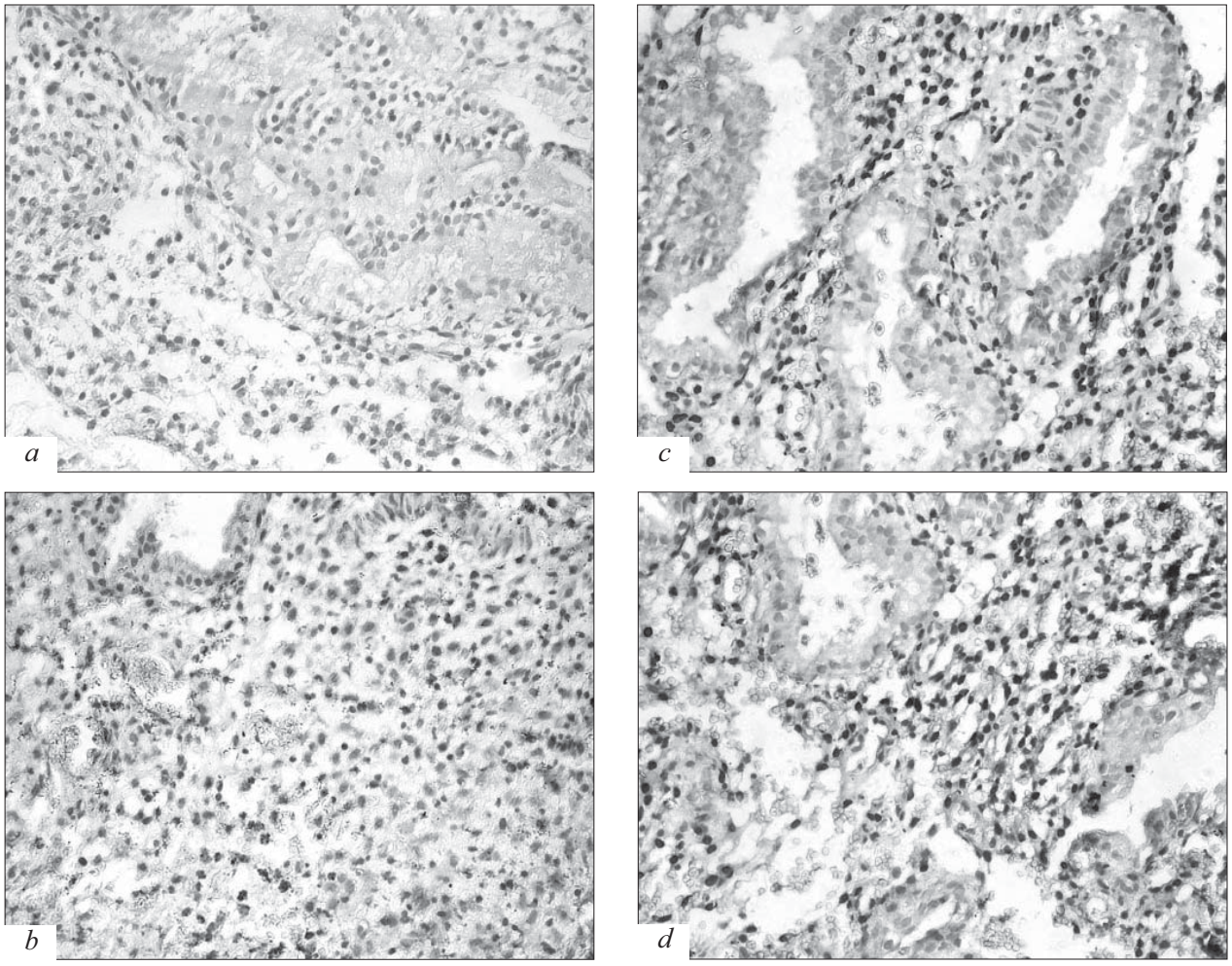

Fig. 1. Expression of progesterone receptors in endometrial cells in HM in the middle of the secretory phase of the menstrual cycle. Immunohistochemical study, two-step streptavidine-biotin method, staining with diaminobenzidene and hematoxylin, ×300. *a*) before therapy: low expression; *b*) normal level; *c*) after metabolic therapy+duphaston: increase in the count of labeled cells; *d*) after metabolic+biphasic hormonal therapy: increase of expression.

Serum concentrations of FSH, LH, estradiol, and progesterone in HM patients before therapy differed significantly from the control; therapy led to positive changes in all groups. The best result was attained in group 3 (Table 1).

The main ultrastructural criteria of the lutein phase of the cycle, evaluated by the control group, were endometrial glandulocyte organelles: 70% cells had intranuclear tubular system, presented by parallel layers of tubular cisterns (Fig. 2, *a*), megamitochondria, and large depositions of glycogen (Fig. 2, *b*), which also emerged in the majority of endometrial epitheliocytes in group 3 as a result of combined metabolic and biphasic hormone therapy (Fig. 2, *c*-*e*). It seems that the secretory phase glandulocytes are in need of rapid hyperproduction of cytoplasmic membranes for preparation of implantation, and hence, the intranuclear tubular system can serve as the marker of endometrial reception.

The glandulocyte apical plasma membrane formed microvilli and solitary cilia (Fig. 2, *f*). The lesser part of epitheliocytes lost the microvilli and protruded into the glandular lumen as pinopodias, regarded as the ultrastructural markers of endometrial reception: they were detected near the blastocyst adhesion [8]. Ultrastructural study of glandular epitheliocytes in groups 1 and 2 detected just small accumulations of glycogen without megamitochondria, this indicating insufficiency of plastic and energy material for full-value initiation of pregnancy under conditions of incomplete secretory transformation of the endometrium.

Hence, evaluation of the effects of various protocols of pre-gestation treatment on the structure and function of the endometrium showed that changes in the endometrium in biopsy specimens from group 1 patients reflected the discrepancy between cyclic

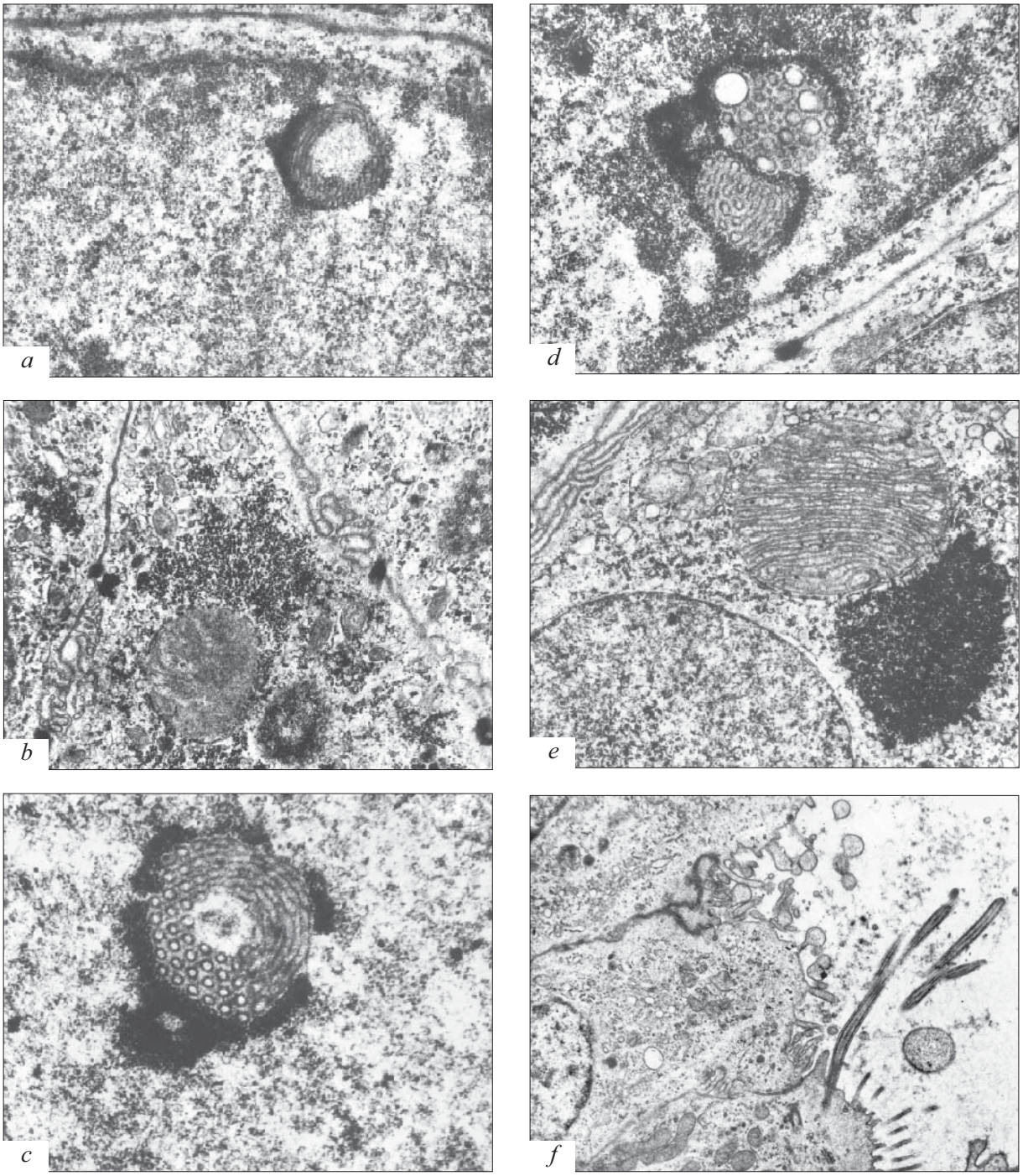

Fig. 2. Ultrastructure of endometrial epitheliocytes in HM in the middle of the secretory phase of the menstrual cycle. Endometrial biopsy: *a*, *b*) normal; *c*-*e*) metabolic+biphasic hormonal therapy; *f*) metabolic therapy. *a*, *b*, *e*, *f*: ×10,000; *c*, *d*: ×20,000. *a*) intranuclear tubular system: complex of tubular cisterns; *b*) accumulation of glycogen and a large mitochondrion in the cytoplasm; *c*) intranuclear tubular system in association with the nucleolar granular component; *d*) doubling of tubular system, tubular structures polymorphism; *e*) giant mitochondrion with numerous parallel cristae, glycogen deposition; *f*) polymorphic microvilli and cilia on the apical surface of an epitheliocyte, numerous ribosomes and small mitochondria in the cytoplasm.

transformations of the endometrium during the middle stage of the secretion phase. Duphaston in parallel with metabolic therapy was more effective, but failed to fully provide the adequate development of the endometrium. Complex pre-gestation therapy (group 3) provided a high level of progesterone receptor expression, corresponding to the parameters in the control group.

The study demonstrated that expression of progesterone receptors, induced by biphasic hormone ther-

apy, can be used as a factor stimulating the secretory function of the endometrium. It promoted the onset and prolongation of gestation in 20% women in group 1, 40% in group 2, and 47% in group 3. These results can be important for further research in reproductive medicine.

REFERENCES

1. N. M. Anichkov, *Arkh. Patol.*, No. 6, 3-8 (2001).
2. V. I. Kulakov, V. N. Serov, and L. V. Adamyan, *Manual of Reproductive Health Protection* [in Russian], Moscow (2001).
3. V. M. Sidelnikova, *Habitual Miscarriage* [in Russian], Moscow (2002).
4. G. T. Sukhikh and L. V. Vanko, *Immunology of Gestation* [in Russian], Moscow (2003).
5. O. K. Khmelnitskii, *Pathomorphological Diagnosis of Gynecological Diseases* [in Russian], St. Petersburg (1994).
6. C. Isaac, J. W. Pollard, and U. T. Meier, *J. Cell. Sci.*, **114**, Pt. 23, 4253-4264 (2001).
7. N. Kittur, G. Zapantis, M. Aubuchon, *et al.*, *Mol. Biol. Cell.*, **18**, No. 6, 2296-2304 (2007).
8. G. Nikas, *Hum. Reprod.*, **14**, Suppl. 2, 99-106 (1999).
9. A. Petersen, U. Bentin-Ley, V. Ravn, *et al.*, *Fertil. Steril.*, **83**, Suppl. 1, 1255-1263 (2005).