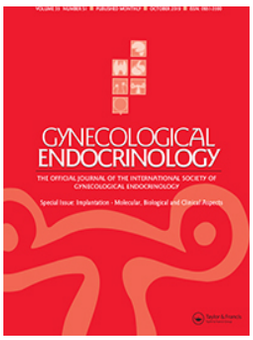

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ORIGINAL ARTICLE

Clinical and laboratory parameters and morphological characteristics of the endometrium in women with impaired fat metabolism and failed IVF attempts

Tatiana V. Lisovskaya[a,b], Tatiana A. Perepletina[a], Ol'ga Y. Sevost' Yanova[c], Elena N. Mayasina[b], Daniil F. Salimov[b] and Anna A. Osipenko[b]

[a]FSBI "Ural Research Institute for Maternity and Childcare" of the Ministry of Health of Russia, Ekaterinburg, Russia; [b]Clinical Institute for Reproductive Medicine, Ekaterinburg, Russia; [c]FGBI of Higher Education "Ural State Medical University" of the Ministry of Health of Russia, Ekaterinburg, Russia

ABSTRACT

Objective: To study clinical and laboratory parameters and morphological characteristics of the endometrium in women with impaired fat metabolism and failed IVF attempts.

Materials and methods: Clinical examination, laboratory tests, morphological analysis, and immunohistochemistry of the endometrium were conducted in 76 patients with different BMI, followed up with infertility and failed IVF attempts. Patients were divided into four groups by body mass index (BMI): 1 group – 17 women with overweight, BMI = 25.0 – 29.9 kg/m^2; 2 group – 15 women with class I obesity, BMI 30.0–34.9 kg/m^2; 3 group – 14 women with class II obesity, BMI 35.0–39.9 kg/m^2; and the control group of 30 women with normal weight, BMI 18.5–24.9 kg/m^2.

Results: Clinical and laboratory analysis revealed menstrual irregularities and hormonal imbalance such as hypoestrogenism. Immunohistochemistry of the endometrium found a significant decrease in the expression of ERα and PR receptors in the glands correlated to increasing BMI.

Conclusion: Pregravid preparation of women with increased BMI and failed IVF attempts has to include life-style modification and weight reduction program to restore normal hormonal status and expression of estrogen and progesterone receptors, prevention of excessive proliferative processes in the endometrium, and improving endometrial receptivity.

KEYWORDS

BMI; endometrium; morphological and immunohistochemical characteristics; IVF program outcomes

Introduction

According to the World Health Organization (WHO), the worldwide prevalence of obesity has been constantly increasing. Over the period from 1975 to 2016 the number of registered cases increased three-fold [1]. Interestingly, that obesity is more common in women compared with men with BMI exceeding the normal limits [1–3]. If these trends continue, by 2025, global obesity prevalence will reach 21% women [1,4].

Research demonstrates the effects of overweight on the women's reproductive health [3,5] and uterine sensitivity for implantation [6,7]. One of the major factors of successful embryo implantation, along with its morpho-functional characteristics, is a synchronized dialog between maternal and embryonic tissues. This so-called endometrial receptivity is related to many hormonal and metabolic factors, as well as metabolic and immunohistochemical characteristics [6].

Considering the data on the poor success rate of IVF programs in women with obesity [8,9], the relations among metabolic, hormonal changes due to obesity and morpho-functional status of the endometrium, and endometrial receptivity in IVF programs are an area of our particular interest.

Materials and methods

We examined 76 reproductive-aged women with impaired fat metabolism, infertility, and failed IVF attempts (from one to three) with blastocyst transfers scored ≥BB grade, according to blastocyst grading system introduced by Gardner in 1999 [10]. Patients' examination included clinical, laboratory tests, and morphological analysis with immunohistochemical staining of the endometrial sections.

According to the WHO classification (1997), the patients were divided into four groups: 1 group – 17 women with overweight, BMI = 25.0–29.9 kg/m^2; 2 group – 15 women with class I obesity, BMI 30.0–34.9 kg/m^2; 3 group – 14 women with class II obesity, BMI 35.0–39.9 kg/m^2; and the control group of 30 women with normal weight, BMI 18.5–24.9 kg/m^2. The median BMI accounted for 27.4 (26.3–28.4) kg/m^2 in group 1; 33.5 (30.4–34.6) kg/м^2 in group 2; 37.4 (35.2–38.4) kg/m^2 in group 3; and 21.2 (19.6–21.8) kg/m^2 in the control group, with a significant between-group difference ($p < .05$). There were no patients with class III obesity included in the study. The duration of infertility on average lasted to 3.8 ± 1.6 years and did not differ significantly between groups, $p > .05$. All studied women had an ovarian reserve preserved (anti-Mullerian hormone, AMH >1.0 ng/ml) and the number of antral follicles of no less than five in each ovary).

Exclusion criteria: age under 18 and over 39 years old; active urogenital infections, male infertility, and patients with endometriosis.

The median age of women in the three study groups and the control group constituted 32.0 (31.5–36.0), 32.6 (30.5–36.4), 32.4 (31.5–36.8), and 31.9 (31.0–37.0), $p > .05$.

CONTACT Tatiana A. Perepletina perepletinata@yandex.ru FSBI "Ural Research Institute for Maternity and Childcare" of the Ministry of Health of Russia, Repina Str., 1, Ekaterinburg 620028, Russia

In order to evaluate the readiness of the endometrium for embryo implantation, we performed morphological analysis and immunohistochemical assessment of the endometrial biopsy specimens received after manual vacuum aspiration on days 7–9 of the typically 29 ± 1 menstrual cycle.

Immunohistochemistry was performed to measure the intensity of brown coloring staining and h-score for progesterone and estrogen receptors (PR, ER-α) in epithelial and stromal cells. The H-score method was used according to the formula: HS = 1a + 2b + 3c, where *a* is the percentage share of poorly stained cells; *b* is the percentage share of moderately stained cells; *c* is the percentage share of strongly stained cells; and 1, 2, and 3 mean the staining intensity expressed in scores. The degree of manifestation of the estrogen and progesterone expression was interpreted as follows: 0–10 scores – lack of expression, 11–100 scores – weak expression, 101–200 scores – moderate expression, and 201–300 scores – pronounced expression. For the κi-67 marker, only the percentage of intensively stained cell nuclei was considered [11].

Additionally, a correlation analysis was conducted to determine the effects of obesity on implantation capacity of the endometrium.

Statistical analysis was performed using the software program for IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA). Methods for estimating the sample mean and standard error are the following: Student's *t*-test – for parametric and Mann–Whitney *U*-test – for nonparametric analysis.

Results

The patients complained of problems related to the reproductive system such as infertility and failed IVF attempts (100% – in all study groups, $p > .05$), heavy periods (23.5% – in group 1; 26.7% – in group 2; 28.6% – in group 3; and 26.7% – in the control group, $p > .05$), painful periods (52.9% – in group 1; 60.0% – in group 2; 64.2 – in group 3; and 50.0% – in the study group, $p > .05$). Besides, women of group 3 complained of uterine bleeding between the periods (metrorrhagia) in 7.1% cases while women in the other groups did not complain of that ($p > .05$). Despite there was no significant difference between groups, the amount of complains related to the reproductive system increased with increasing BMI.

According to the international classification of diseases (ICD-10), infertility caused by anovulation (N97.0) prevailed among the infertility causes in women with overweight and class I and II obesity and constituted 47.1%, 53.3%, and 57.2%, respectively, $p < .05$, while in women with normal body weight, it was 26.7%. In the control group, tubal infertility (N97.1) was revealed significantly more often (43.3%) compared with the study groups where it was found in 23.5% – in group 1, 13.3% – in group 2, and 7.1% – in group 3. There were no between-group differences in uterine infertility (N97.2) and unexplained infertility (N97.9) (Figure 1).

There was a little increase in aspartate aminotransferase (AST) levels up to 45.0 (26.25–67.47) units per liter (U/L) and alanine aminotransferase (ALT) levels up to 50.9 (24.97–79.5)

Table 1. Estradiol and progesterone levels in women with impaired fat metabolism and failed IVF attempts, Me (25–75).

	Group 1 ($n = 17$)	Group 2 ($n = 15$)	Group 3 ($n = 14$)		
Parameter	1	2	3	Control group ($n = 30$)	p Value
Estradiol on days 3–5 of the menstrual cycle, nmol/L	81.5 (68.6–83.3)	73.3 (25.4–82.0)	49.5 (39.5–53.6)	158.0 (78.0–183.0)	$p_{1,2} = .030$ $p_{1,3} = .031$ $p_{1,4} = .028$ $p_{2,3} = .022$ $p_{2,4} = .024$ $p_{3,4} = .018$
Progesterone on days 22–24 of the menstrual cycle, nmol/L	14.2 (12.1–14.1)	14.7 (10.7–17.2)	14.5 (12.7–15.2)	14.5 (12.3–15.6)	$p > .05$

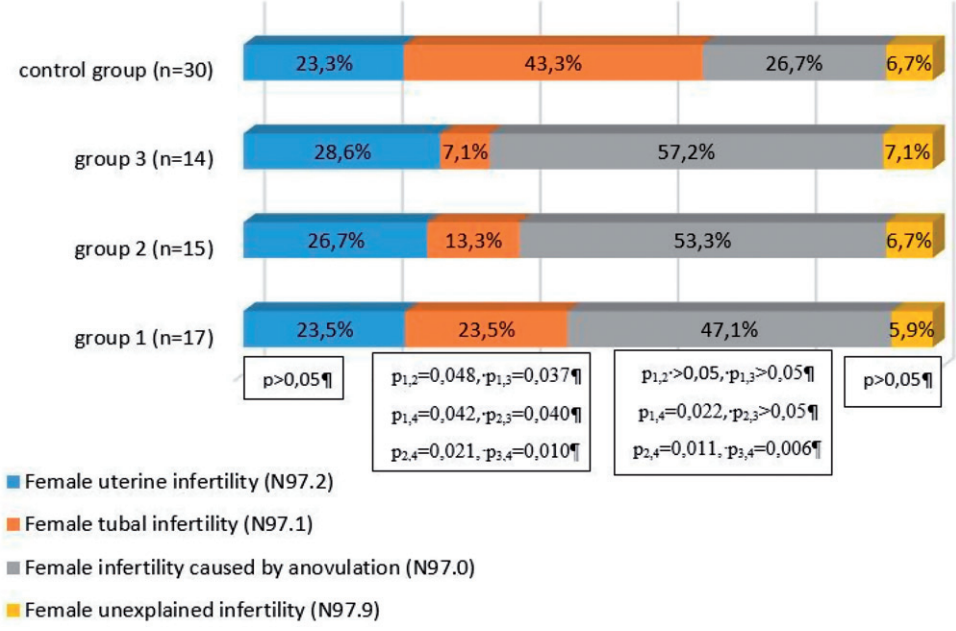

Figure 1. Infertility in women with impaired fat metabolism and failed IVF attempts, by reasons, and BMI.

U/L in the biochemistry test in women with class II obesity; however, it was significantly different from AST and ALT levels in women with overweight and class I obesity whose parameters were within reference values: 25.8 (16.0–30.0) U/L and 21.1 (12.4–28.1) U/L, 28.6 (26.0–32.0) U/L, and 24.3 (22.1–38.3) U/L, $p < .05$ and did not differ from those in women with normal body weight: 18.3 (14.9–27.1) U/L and 18.0 (15.3–29.4) U/L, $p < .05$.

Considering the effects of estrogen and progesterone on endometrial preparation for implantation [6], we analyzed the hormonal level in our patients. The results of the analysis demonstrated a decrease in estradiol in the first phase of the menstrual cycle in overweight women, more evidenced in women with class I and II obesity: 81.5 (68.6–83.3), 73.3 (25.4–82.0), and 49.5 (39.5–53.6) nmol/L, $p < .05$ compared with the control group – 158.0 (78.0–183.0) nmol/L, $p < .05$. Progesterone levels on days 22–24 of the menstrual cycle in all groups were within reference values and did not differ significantly between groups: $p > .05$ (Table 1).

In most women with normal body weight, histological examination showed that glandular epithelium was in the early proliferation phase (83.3%), the rest of the women had stromal infiltration with solitary lymphoid cells according to the cycle phase. In the study groups, morphological changes were more evident with increasing body weight. In overweight women in group 1, histological examination data in 41.2% was consistent with an early proliferation phase without any signs of stromal inflammatory reaction whereas in groups 2 and 3 such histological data were found in 26.7% and 14.3%. In 29.4% women in group 1, 26.7% in group 2, and 21.4% in group 3, the endometrial glandular epithelium was consistent with the proliferation phase with a different degree of moderate stromal lymphoid infiltration. Histology examination was not consistent with the cycle phase in 17.6% women in group 1, 20.0% in group 2, and 21.4% in group 3. Simple endometrial glandular hyperplasia with large and cystic dilated glands was detected in 11.8% women in group 1, 20.0% in group 2, and 28.6% in group 3. The proliferative process with signs of chronic inflammation was revealed only in groups with class I and II obesity: 6.6% and 14.3%, respectively.

Immunohistochemistry for paraffin-embedded tissue sections found that levels of expression of both ERα and PR receptors in the endometrial glands prevailed over those parameters in the stroma [11], as well as was decreased with increasing BMI (Figure 2). The lower level of both ERα and PR expression determined in the study groups compared with the control group of women with normal body weight was obviously related to a more pronounced endometrial fibrosis in women with increased BMI. Ki-67 immunohistochemistry evidenced of proliferation process in the endometrium found that elevated expression was predominantly found not in the stoma but in the glandular epithelium. Ki-67 expression in the glands was elevated with

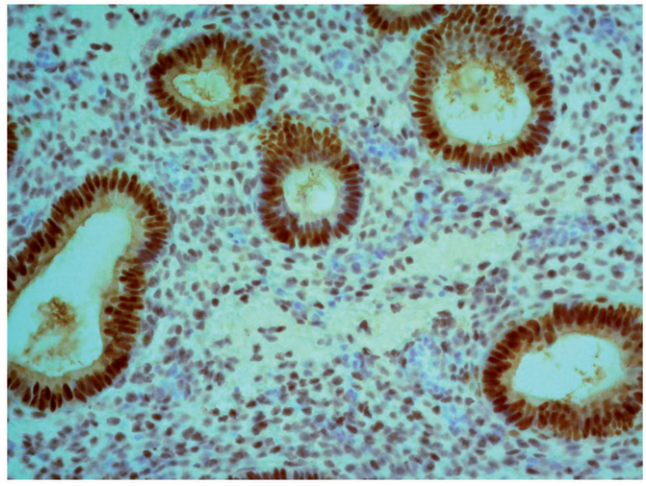

Figure 2. Patient P., 35 years old, class II obesity, 2 failed IVF attempts. Day 7 of the menstrual cycle. Immunohistochemical assay for progesterone receptors, original magnification ×200. Intensive staining of glandular epithelium, in the stroma – small quantity of cells with mitotic activity.

Table 2. Immunohistochemical characteristics of the endometrium in women with impaired fat metabolism and failed IVF attempts, Me (25 – 75).

		Group 1 ($n = 17$)	Group 2 ($n = 15$)	Group 3 ($n = 14$)	Control group ($n = 30$)	
Characteristics	Localization	1	2	3	4	p value
PR (scores)	In stroma	145.0 (131.5–202.5)	117.0 (102.5–230.0)	74.0 (62.5–200.0)	165.0 (110.0–298.0)	$p_{1,2} > .05$ $p_{1,3} = .012$ $p_{1,4} > .05$ $p_{2,3} = .032$ $p_{2,4} = .018$ $p_{3,4} = .009$
	In glands	155.0 (132.5–176.0)	129.0 (104.0–156.5)	99.0 (64.0–116.5)	175.0 (120.0–298.0)	$p_{1,2} = .023$ $p_{1,3} = .022$ $p_{1,4} = .024$ $p_{2,3} = .022$ $p_{2,4} = .024$ $p_{3,4} = .018$
ER-α (scores)	In stroma	156.0 (111.0–175.0)	152.0 (147.5–186.0)	146.0 (104.0–158.0)	275.0 (234.0–298.0)	$p > .05$
	In glands	272.0 (161.0–295.0)	196.0 (194.0–198.0)	166.0 (94.0–198.0)	280.0 (275.0–297.0)	$p_{1,2} = .036$ $p_{1,3} = .021$ $p_{1,4} > .05$ $p_{2,3} > .05$ $p_{2,4} = .034$ $p_{3,4} = .028$
Ki 67 (%)	In stroma	11.0 (5.5–33.5)	13.6 (2.5–29.5)	23.6 (12.5–28.5)	11.8 (6.0–16.5)	$p > .05$
	In glands	21.4 (19.2–28.0)	32.6 (29.2–38.0)	69.3 (47.0–80.0)	18.2 (12.0–28.0)	$p_{1,2} = .013$ $p_{1,3} = .002$ $p_{1,4} = .014$ $p_{2,3} = .012$ $p_{2,4} = .016$ $p_{3,4} = .008$

increasing body weight: 21.4 (19.2–28.0) in group 1; 32.6 (29.2–38.0) in group 2; 69.3 (47.0–80.0) in group 3; and 18.2 (12.0–28.0) in the control group, $p < .05$ (Table 2).

The correlation analysis of clinical, laboratory, and morphological parameters of the endometrium revealed a significant positive correlation between BMI and failed IVF attempts in the groups of women with class I and II obesity ($r = 0.785$, $p = .028$ and $r = 0.883$, $p = .018$, respectively). The correlation analysis among clinical, laboratory, and immunohistochemistry parameters detected a significant negative correlation between serum WBC count and the level of PR receptor expression in the glands: $r = -0.500$, $p = .008$ in group 1, $r = -0.575$, $p = .002$ in group 2, and $r = -0.465$, $p = .003$ in group 3, as well a significant positive correlation between BMI and proliferation marker Ki-67 in the endometrial glands: $r = 0.706$, $p = .026$ in group 1, $r = 0.749$, $p = .006$ in group 2, and $r = 0.805$, $p = .002$ in group 3. In women with class I and II obesity, there was a significant negative correlation between serum glucose and ERα expression in the glands: $r = -0.379$, $p = .038$ and $r = -0.424$, $p = .020$, respectively; between serum glucose and proliferation marker Ki-67 in the endometrial stroma: $r = -0.370$, $p = .42$ and $r = -0.376$, $p = .046$, respectively.

Conclusion

With increasing BMI, reproductive-aged women had more frequent menstrual dysfunctions, a significant decrease in estradiol in the first phase of the menstrual cycle while maintaining progesterone levels on days 22–24 of the menstrual cycle within the reference values. However, that fact did not exclude excessive proliferative processes of the endometrium which was evidently one of the reasons for reduced endometrial receptivity and impaired implantation in IVF cycles.

Disclosure statement

The authors of the article have no conflicts of interests.

Ethical approval

The research protocol was approved by the Independent Ethical Committee of Clinical Institute for Reproductive Medicine", LLC (# 06, dated June 4 2018)

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