



The Impact of Endometrial Hyperplasia without Atypia on *In Vitro* Fertilization Outcome

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Abstract

Context: A role of endometrial hyperplasia in efficacy of assisted reproductive technology remains unclear as do involvement of local antioxidant defense system and vitamin D serum level.

Aim: To determine whether endometrial hyperplasia without atypia impacts *in vitro* fertilization outcome as well as highlight a role of local antioxidant defense system and 25-hydroxycalciferol serum level as possible markers for ART efficacy in infertile women with endometrial hyperplasia.

Setting and design: Two-stage observational non-interventional study with 2000 clinical cases analyzed during retrospective stage and 250 participants on prospective stage.

Materials and methods: Pregnancy rate was assessed to determine an influence of endometrial hyperplasia without atypia on *in vitro* fertilization outcome. Local antioxidant defense level was registered by superoxide-dismutase activity detection and total antioxidant capacity measurement in endometrial tissue prior to embryo transplantation. 25-hydroxycalciferole serum concentration was determined by quantitative enzyme-linked immunosorbent assay method.

Statistical analysis used: We applied “Chi-square” criterion to comparatively assess *in vitro* fertilization outcome. The normality of the variance distribution was determined using one-way Analysis of Variance (ANOVA), while Newman-Keuls criterion was used to intergroup assessment.

Results: Pregnancy rate among women with endometrial hyperplasia without atypia undergone *in vitro* fertilization was lower than in control group largely due to results obtained in subgroup of women older than 40. Negative outcome associates with depletion of antioxidant system potency in hyperplastic endometrium and decrease of vitamin D serum level.

Conclusion: Superoxide-dismutase activity in endometrial tissue and local total antioxidant capacity along with vitamin D serum level may be considered as potential biomarkers of fertilization outcome in endometrial hyperplasia without atypia.

Keywords: *In vitro* fertilization outcome; Endometrial hyperplasia without atypia; Superoxide-dismutase; Total antioxidant capacity; 25-hydroxycalciferol

Key Messages

Endometrial hyperplasia without atypia worsens *in vitro* fertilization outcome in women with tubal infertility. Negative outcome associates with depletion of antioxidant system potency in hyperplastic endometrium and decrease of 25-hydroxycalciferole serum level.

Introduction

At present, hyperplastic processes in endometrium are considered as a precancerous endometrial condition with a different degree of malignancy probability [1,2]. The primary etiological factor in the formation of the pathological process is suggested to be long-lasting hyperoestrogenism, which develops against the background of relatively low concentrations of endogenous progesterone. Although the peak of Endometrial Hyperplasia (EH) incidence occurs in women between the ages of

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50 and 60, nevertheless, the disease incidence in younger age groups is significant [3]. Current opinions consider following risk factors for the development of the disease: long-term an ovarian syndrome with normal level of gonadotropins, increased levels of endo- and exogenous oestrogen, and obesity [4]. According to the modern classification revised in 2014, the disease is divided into EH without atypia and EH with atypia [5]. The main diagnostic method is targeted biopsy of the endometrium with morphological verification of the diagnosis, since other diagnostic methods do not allow excluding the presence of a malignant process and possess a number of limitations in such characteristics as sensitivity and specificity [6].

Up to now, there is no strict evidence of the negative effect of EH without atypia on the Assisted Reproduction Technologies (ART) outcome. Scientific publications emerged in recent years contain very ambiguous information on this issue, the studies described in them have been made on a small sample and, moreover, usually included women with endometrial malignancies [7,8]. In one of the studies conducted by Gaducci et al. [9] the authors have shown that the completion of hormonal therapy, accompanied by clinical and laboratory effects, has led to the successful implementation of fertile function, both naturally and with the use of *In Vitro* Fertilization and Embryo Transfer (IVF ET) protocols [9].

Involvement of oxidative stress reactions and free radical generation has been shown for EH development in fertile women and IVF ET outcome in infertile females [10,11]. Cinar et al. [10] have demonstrated high predictive potency of catalase, malondialdehyde and xanthine oxidase in EH development. Nishihara et al. [12] have reported depletion of total antioxidant capacity and different deviation of glutathione level in infertile women in dependence on gynecological cause of infertility. Vitamin D both serum and follicular fluid levels attract particular attention of scientists since its role in reproduction has been demonstrated [13,14]. Thus, it has been shown that vitamin D and its biologically active metabolites regulated sex steroid hormones secretion both in follicular and placental tissue, influenced gonadotropins' expression [15,16]. However, studies on effectiveness of ART methods in women of reproductive age with EH without atypia, identification of molecular and biochemical markers and predictors of IVF ET success have not been carried out yet, which determines the undoubted relevance and timeliness of this work.

The main aim of the study is to determine whether EH without atypia impacts IVF ET outcome as well as highlight a role of local antioxidant defense system and 25-hydroxycalciferol serum level as possible markers for ART efficacy in infertile women with EH.

Methods and Material

This study is observational clinical non interventional study consisted of retrospective and prospective stages.

Selection and description of participants

On retrospective stage of the study 1886 clinical cases of women with tubal-peritoneal infertility underwent IVF ET protocols at Obstetrics and Gynecology University Clinic of Sechenov University between 2014 and 2017 were collected and analyzed. To achieve the main aim of the study we selected only cases of women with histological proven EH without atypia or ones without hyperplasia. We excluded all cases of another endometrial pathology such as endometriosis and endometritis to avoid possibility of statistical bias. IVF ET success was registered in case of pregnancy (successful

pregnancy rate) confirmed by ultrasound examination four weeks after ET. The prospective stage of the study involved 150 women of reproductive age with tubal infertility and EH without atypia, which was established according to the results of hysteroscopy with guided biopsy (RCOG/BSGE Joint Guideline, 2016) during preparation for IVF ET at Obstetrics and Gynecology University Clinic of Sechenov University from October 2017 till August 2018.

Inclusion criteria were as follows: age of patients from 18 to 45 years old, voluntary informed consent to participate in the study, infertility of tubal origin, EH without atypia (for the main group) diagnosed for the first time and verified by targeted biopsy and subsequent morphological examination of endometrial tissue specimens, no more than 2 unsuccessful IVF attempts. Exclusion criteria for the study were the presence of acute and chronic inflammatory diseases of the uterus during the last 12 months, endometriosis of any location, the presence of acute or chronic infectious diseases (including viral hepatitis and HIV infection) during the last 3 months, chronic somatic diseases (arterial hypertension, kidney and liver disease). In control group we enrolled 100 infertile women without hyperplastic changes in endometrium who met inclusion criteria in the absence of exclusion criteria, and voluntarily agreed to participate in the study.

Ethics

The Study Protocol (No. 1275) was reviewed and approved by Sechenov University Ethics Committee (No. of review 128, date of Review meeting April 12, 2017). Expressed informed consent was received from each of the study participant. All medical procedures, services and manipulations were free of charge for the women due to Russian Government financial support of ART programmers in the country.

IVF protocol

We induced ovulation with gonadotropins-releasing hormone antagonists (Diphereline[®], Beaufor Ipsen Pharma, France) according to the standard method. Oocyte fertilization was carried out by IVF. On the 5th cultivation day selective transfer of 1 blastocyst of 4 to 5 quality class was performed.

Methods of local oxidative stress measurement

We assessed oxidative stress activity in visually intact and hyperplastic endometrial specimens by Superoxide-Dismutase (SOD) activity detection and Total Antioxidant Capacity (TAC) measurement. Endometrial tissue was taken during hysteroscopy examination at the second half of phase I of menstrual cycle (from day 6 to 12) prior to embryo transplantation. SOD activity was determined as previously described [17]. TAC was measured by Fe-induced Chemiluminescence (CL) method as previously described [18].

Serum 25-hydroxycalciferol concentration determination

25-Hydroxycalciferol (25-OH-D) concentration in blood serum was carried out by quantitative Enzyme-Linked Immunosorbent Assay (ELISA) method using a standard set of reagents (DGR Tech Systems, Russia). Blood samples (5 ml) were taken between day 2 and 6 of menstrual cycle in the morning hours, on an empty stomach, under conditions of physiological rest from the cubital vein. According to the Institute of Medicine and the Endocrine Society Clinical Practice Guideline Vitamin D deficit was defined when 25-OH-D serum concentration was less than 20 ng/mL. We considered the range of 25-OH-D serum level within 20 ng/mL and 30 ng/mL as

Table 1: Age-related IVF ET success rate in infertile women with EH without atypia in comparison with infertile female without endometrial pathology.

Group of women	Age	Confirmed pregnancy (%)	P-value
Subgroup A1, n=714	18-40	304 (42.6)	0.126
Subgroup B1, n=673	18-40	315 (46.8)	
Subgroup A2, n=218	>40	27 (12.4)	0.002
Subgroup B2, n=281	>40	70 (24.9)	
Group A, n=932	All	331 (35.5)	0.047
Group B, n=954	All	385 (40.4)	

Note: *significant difference when compared with group B or subgroup B2 (Chi-square test)

replete-insufficient [19,20].

Statistics

Statistical processing of the collected data was carried out using SPSS (IBM Corp., USA, version 16.0) [21]. We applied “Chi-square” criterion to comparatively assess IVF ET outcomes in main and control groups on both retrospective and prospective stages of the study. Collected data was presented as Mean Value (M) ± Square Deviation (SD). The normality of the variance distribution was determined using one-way analysis of variance (ANOVA), while Newman-Keuls criterion was used to intergroup assessment.

Result

We assessed retrospectively if HE without atypia influenced IVF ET protocols efficacy in women with tubal infertility (Table 1). All cases were divided into main group (group A, n=932, women with EH without atypia) and control group (group B, n=954, infertile women without endometrial pathology). In turn, each group was then subdivided according to the women’s age into two subgroups. Cases of females of 18 to 40 years old were collected into A1 and B1 subgroups whereas subgroups A2 and B2 comprised cases of older ones. Pregnancy rate was measured comparatively for each pairs of subgroups (A1 and B1, A2 and B2, respectively), and for whole groups A and B. 304 women of subgroup A1 (42.6%) became pregnant vs. 315 (46.8%) in subgroup B1, which difference was insignificant (p=0.126). Among women of older age 27 (12.4%) infertile females of subgroup A2 achieved favorable result of IVF ET whereas they were 70 (24.9) with confirmed pregnancy in subgroup B2. The intergroup difference was statistically significant (p=0.002) as it was when compared both the groups (A and B) in the whole (p=0.047).

On the prospective stage of the study local oxidative stress activity in endometrial tissue and 25-OH-D level in participant’s blood serum were assessed. Main and control groups (C and D, respectively) in accordance with IVF ET success were divided into two subgroups as followed: both C1 and D1 comprised cases with achieved pregnancy; C2 and D2 otherwise. Pregnancy rate in both main and control groups reproduced aforementioned results obtained on retrospective stage of the study (Table 2).

SOD activity in intact endometrial tissue of control group participants averaged 10.9 IU/g ± 0.2 IU/g in subgroup D1 and 11.4 IU/g ± 0.4 IU/g in subgroup D2 (Table 2). In infertile women with endometrial hyperplastic process (main group) SOD activity was determined both in the affected tissue and in the visually unchanged endometrium. In the main group of patients with successful IVF ET (subgroup C1) an increase in the enzyme activity in visually unchanged endometrium was observed (14.6 ± 0.3, p=0.003) with an absence of differences in its activity in the hyperplasia zone (11.3

Table 2: SOD activity and TAC capacity (Mean ± SD) in endometrial tissue of infertile women with or without hyperplasia of endometrium.

Group of women	n	P-value	Zone of endometrium	SOD, IU/g	TAC capacity
CL, imp/s × 10 ³					
Subgroup C1	58	0.043¶	Visually intact	14.6 ± 0.3	25.6 ± 1.4
			Hyperplastic	10.9 ± 0.2*	13.4 ± 0.7*
Subgroup D1	43		Visually intact	11.3 ± 0.4	16.7 ± 1.3††
Subgroup C2	92		Visually intact	6.8 ± 0.3†	14.2 ± 0.5††
			Hyperplastic	4.3± 0.2††	8.0 ± 0.4††
Subgroup D2	57		Visually intact	11.4 ± 0.4	15.9 ± 1.1††

Note: *p<0.05 when compared with visually intact endometrium of the same subgroup (ANOVA); †p<0.05 when compared with visually unchanged tissue of subgroups C1 and D1 (Newman-Keuls criterion); ††p<0.05 when compared with related zone of subgroup C1 (ANOVA); ¶ chi-square test

IU/g ± 0.4 IU/g). In endometrial tissue of females of subgroup C2, contrast changes in SOD activity were found when compared with subgroup C1 (Table 2). We registered more than twofold decrease in SOD activity in visually unchanged endometrial tissue (6.8 IU/g ± 0.3 IU/g), and even deeper stagnation its tissue level (4.3 IU/g ± 0.2 IU/g) in hyperplastic endometrium.

We found that the fluctuations in TAC rate of intact endometrial tissue of women in subgroup D1 averaged (16.7 ± 1.3 × 10³ imp/s). The results fully corresponded with TAC level measured in subgroup D2 (15.9 ± 2.1 × 10³ imp/s, p=0.86) (Table 2). In subgroup C1 CL intensity detected in visually unchanged endometrium was two times higher (25.6 imp/s ± 1.4 imp/s × 10³) than in hyperplastic tissue (13.4 imp/s ± 0.7 imp/s × 10³, p=0.001) and significantly higher than in related zone of subgroup D1 (p=0.005). Hyperplastic endometrial tissue of women in subgroup C2 was characterized by decreased TAC level in comparison with subgroup C1 (8.0 imp/s ± 0.4 imp/s × 10³, p=0.002), while CL intensity measured in visually intact endometrium (14.2 imp/s ± 0.5 imp/s × 10³) remained at subgroup D2 level (p=0.24). 27.9% of subgroup D1 participants and 29.8% of subgroup D2 women were deficient of 25-OH-D serum level (Table 3). 67.4% and 63.2% of females in subgroups D1 and D2 respectively had replete-insufficient the vitamin serum level. According to vitamin D serum level scale participants of main group were ranged significantly different. 32.8% of them in subgroup C1 were 25-OH-D deficient vs. 44.6% in subgroup C2 (p=0.039). 25-OH-D serum level in 41 women of subgroup C2 had lowest value (7.2 ng/mL ± 0.8 ng/mL, p=0.01) when compared with both subgroups C1 and D2.

Discussion

It’s still remained disputable whether EH without atypia influenced such favorable IVF ET outcome as pregnancy rate or birth rate. Different and even contrast opinions have been published for recent years. In current study we have shown age-related interaction between endometrial hyperplastic pathology and pregnancy rate. In particular, it turned out that pregnancy rate in subgroup of 18 to 40-year-old women with EH without atypia underwent IVF ET was the same as in subgroup of infertile females without endometrial pathology. In contrast, in older women’s subgroup IVF ET efficacy was significantly lower in females with EH without atypia. These results made the differences between main and control age-independent groups statistically significant.

There is no doubt now that oxidative stress plays an important role in pathogenesis of endometrial pathology such as endometriosis,

Table 3: 25-OH-D serum level in infertile women with EH without atypia and females with tubal infertility at absence of endometrial pathology underwent IVF ET.

Scale of vitamin D serum level		Main group		Control group	
		Subgroup C1 n=58	Subgroup C2 n=92	Subgroup D1 n=43	Subgroup D2 n=57
Deficient	n (%)	19 (32.8%)	41 [†] (44.6%)	12 (27.9%)	17 (29.8%)
<20 ng/mL	M ± SD	11.9 ± 1.2	7.2 ± 0.8 ^{††}	13.5 ± 1.6	12.2 ± 1.1
Replete-insufficient	n (%)	36 (62.1%)	47 (51.1%)	29 (67.4%)	36 (63.2%)
20 ng/mL to 30 ng/mL	M ± SD	25.2 ± 1.8	23.7 ± 1.6	26.8 ± 1.5	24.5 ± 2.3
Normal	n (%)	3 (5.1%)	4 (4.3%)	2 (4.7%)	4 (7%)
>30 ng/mL	M ± SD	46.9 ± 2.1	44.6 ± 2.0	45.7; 51.3	46.9 ± 2.1

Note: [†]p=0.039 when compared with subgroup C1 (chi-square test); ^{††}p<0.05 when compared with subgroup C1 and D2 (Newman-Keuls criterion)

endometritis, EH, malignancies of endometrium [22]. On the other hand, dysfunction of local antioxidant defense system influences endometrial receptivity and ultimately affects IVF outcome [10,11,22]. Though we have not found differences in SOD activity and TAC level in endometrial tissue of infertile women with successful IVF ET and patients who did not achieve favorable ART result, there has been detected significant depletion of SOD activity in hyperplastic and visually intact endometrium along with decrease of TAC level in hyperplastic zones in infertile women with negative IVF ET outcome.

A number of experimental and clinical studies conducted recently have shown the crucial role of vitamin D serum concentration in the process of carrying a foetus, the interaction of the foetus and the mother's body [13,15]. Results obtained in 2014 by a team of researchers led by Paffoni were very interesting [16]. The authors have shown that the probability of successful implementation of IVF protocol was determined by the level of the 25-OH-D in the blood serum. Thus, in women aged 18-42 years with the vitamin concentration below 20 ng/mL, the frequency of positive results was about 20%, whereas in 31% of cases of the same age females with 25-OH-D level in serum above 20 ng/mL ART successfully accomplished with the onset of pregnancy. At the same time, Banker et al. [14] have demonstrated that Vitamin D deficiency led to lower reproductive outcome, though not statistically significant and, thereby, did not have a negative influence on *in vitro* fertilization-intracytoplasmic sperm injection outcomes. In our study we have shown absence of association between 25-OH-D serum level and IVF ET outcome in group of infertile women without EH, but average Vitamin D serum concentration was significantly lower in subgroup of patients with EH without atypia and IVF ET bad outcomes.

In summary, EH without atypia worsens *in vitro* fertilization outcome in women with tubal infertility. Pregnancy rate among women with endometrial hyperplasia without atypia undergone *in vitro* fertilization was lower than in control group largely due to results obtained in subgroup of women 40 and more years old. Superoxide-dismutase activity in endometrial tissue and local total antioxidant capacity along with 25-hydroxycalciferol serum level may be considered as potential biomarkers of fertilization outcome in endometrial hyperplasia without atypia.

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