THE NEW ARMENIAN MEDICAL JOURNAL
Vol.13 (2019), No 2, p. 87-95

THE PREDICTIVELY FAVORABLE FACTORS OF CLINICAL PREGNANCY AT FROZEN EMBRYO TRANSFER PROTOCOLS IN PATIENTS WITH REPEATED IMPLANTATION FAILURES

Sulima A. N.[1]*, Voronaya V. V.[1], Davydova A. A.[2], Rybalka A. N.[1], Dizha M. A.[1]

V.I. Vernadsky Crimean Federal University, Medical Academy Named after S.I. Georgievsky; Simferopol, Russian Federation

[1]Department of obstetrics, gynecology and perinatology
[2]Department of pathological anatomy with sectional course

Received 03.11.2018; accepted for printing 22.02.2019

Abstract

This article is devoted to a problem of forecasting of the frozen embryo transfer results at patients with repeated implantation failures (more than two) and to identification of the favorable factors of clinical pregnancy.

The research included retrospective and prospective stages.

At a retrospective stage for the identification of repeated implantation failures risk factors, 200 outpatient cards of the patients undergoing of artificial reproductive technologies were analyzed. 100 patients had repeated implantation failures at frozen embryo transfer in the anamnesis, 100 patients had only one successful frozen embryo transfer, and pregnancy occurred.

The influence of various schemes of replacement hormonal therapy on endometrium and the results of frozen embryo transfer was studied at prospective stage. Patients of a retrospective stage with repeated implantation failures which depending on the estrogen medication (an estradiol valerate or 17β-estradiol) as a part of replacement hormonal therapy were divided into two equivalent groups and entered a prospective stage.

Predictively favorable factors of clinical pregnancy in frozen embryo transfer cycles at patients with the repeated implantation failures are two and more gynecologic diseases in the anamnesis; two and more extragenital diseases; two and more pelvic organs surgeries in the anamnesis; two and more intrauterine interventions in the anamnesis; two and more factors of infertility and also absence of pathologic morphological changes at endometrium after miscarriages in the anamnesis and duration of infertility is less than 10 years. Also, the expressed expression of endometrium α-estrogen receptors and a marker of proliferative activity of endometrium (Ki-67), according to an immunohistochemical research in an early proliferative phase of a previous menstrual cycle to frozen embryo transfer (from 9 to 11 day) using the replaceable hormonal therapy is evidenced by the clinical pregnancy.

Identification of repeated implantation failures risk factors at the patients undergoing of frozen embryo transfer is an unfortunate factor of clinical pregnancy. The expressed expression of endometrium α-estrogen receptors and a marker of proliferative activity of endometrium (Ki-67) are associated with increasing of clinical pregnancy rate in frozen embryo transfer protocols at the analyzed patients' group. Estradiol blood level does not correlate with the level of α-estrogen receptors expression and Ki-67 marker in endometrium.

Keywords: *repeated implantation failures, risk factors, frozen embryo transfer protocols, 17β-estradiol, immunohistochemistry, endometrium receptivity.*

Introduction

In 2016, the frequency of infertility rate at married couples in the Russian Federation reached 16.0%, having exceeded thereby the level, critical for social population policy of the state, established by World Health Organization in 15.0%. According to Scientific center of obstetrics and gynecology of the Russian Ministry of Health, in Russia 7-8 million Russian women and 3–4 million men are infertile today. Gender analysis of infertility's structure established up to 45% falls to the share of women and 40% for a share of men [*Dzhamaludinova A. F., Gonyan M. M., 2017*].

Address for Correspondence:

Anna N Sulima, D. Med. Sc., Professor
Department of Obstetrics, Gynecology and Perinatology.
Medical academy named after S. I. Georgiyevsky,
8 Vorovsky St., Simferopol 295017, Republic of Crimea
E-mail: gsulima@yandex.ru.

Now the Government of the Russian Federation accepted a number of measures directed to overcoming current situation among which there is an increase of the artificial reproductive technologies (ART) in structure of a medical care to the married couples having infertility. Because of implementation of this policy, there is annual increasing of total number of the carried-out ART cycles which number according to the Russian Association of Human Reproduction was 113976 in 2016, having exceeded thereby indicators of 2015 for 8.5%, and 2014 – for 22.2%. The reference is not only increase in number of the ART programs, but also change of their structure with gradual increase in a share of programs with use of the thawed embryos (20.3% in 2014, 22.9% in 2015 and 25.8% in 2016) [*Korsak V. S., 2018*]. Increasing of frozen embryo transfer protocols' numbers with use of the thawed embryos displays advantages of "freeze all" policy, namely safety, in view of reduction of complications' risk [*Pandey S. et al., 2012],* and cost efficiency [*Roque M. et al., 2015*].

Despite the expressed progress in this direction, effectiveness the program of thawed embryo transfers remains approximately at one level the last years. In 2014 the pregnancy rate counting on a cycle was 36.9%, on thawed embryo transfer – 37.8%. In 2015 these indicators were 38.6% and 39.9% respectively, and in 2016 - 38.5% and 40.0% [*Korsak V. S., 2018*]. Despite of existence of a positive trend in effectiveness of FET protocols, the achieved results are not enough for a solution of the infertility problem and stabilization of a demographic situation, the number of "test tube baby" was only 0.07% of total number of the born children in 2016. Insufficiently high performance of the ART cycles at annual increase in their quantity causes increasing number of the patients repeated addressing for the treatment. The number of this category of patients, according to some information, makes from 40.0 to 45.0% [*Simon A., Laufer N., 2012*]. Knowledge accumulated in recent years and a large number of observations allows drawing conclusions about need of selection of these patients in separate group and their careful analysis. Most of women of this group distinguishes community of neuroendocrinal regulation disorders of reproductive function [*Simon A, Laufer N, 2012; Nosenko E et al., 2013*] and also morphological and functional inferiority of endometrium [*Gajdukov S. N. et al., 2013*], demanding of corrections and artificial model of cyclic changes in endometrium by preparation for thawed embryo transfer with using of hormonal replacement therapy (HRT) [*Ruiz-Alonso M. et al., 2013*]. Now as an estrogen component of HRT, a number of estradiol drugs is used, which various on their bio-identity to natural estrogen and metabolism.

The key factor defining effectiveness of the ART programs in FET with selectively chosen blastocyst is its adequate preparation of endometrium [*Vartanyan E. V., 2013*]. According to literature, control hysteroscopies with the subsequent endometrial currettage and its histology at these patients show changes in endometrium at 24-62.0% cases [*Mazur M. T., Kurman R. J., 2005; Nosenko E. N. et al., 2013*]. Quite often, uneffectiveness of medical correction results in need to pass to the program of surrogacy.

The condition of endometrium receptor state is reflection of the complex and multiple factor processes in the conditions of its preparation with using of HRT in FET protocols. On the one hand, hormonal drugs have significant effect on the receptors' system. At the same time, with another – disorders in the endometrium receptors, against the background of these or those acquired pathological states can result in its insufficient susceptibility to exogenous hormonal influence [*Crum C. P., 2006, Paulson R. J., 2011*]. Data on ratios between indexes of an expression of receptors of endometrium to estrogen, various on chemical structure, at its various schemes of preparation with using of HRT in FET protocols are contradictory and fragmentary.

Thus, the aim of this study was to increase the pregnancy rate at frozen embryo transfer protocols in patients with the repeated implantation failures.

Material and methods

The research design was presented by two stages: retrospective and prospective. Inclusion criteria in this research were: age from 24 to 45 years; duration of infertility from 3 to 16 years; absence of genetic pathology at the woman and the partner; preparation of endometrium for transfer of the thawed embryo with use of HRT; transfer of one thawed embryo at a stage of a blastocyst, good and excellent quality (3-5 BB, BA, AB, DD) ac-

cording to classification of Gardner D. K., Schoolcraft W. B. (1999) [*Schoolcraft W. B., Gardner D. K., 2001*]. According to above-stated criteria, 200 patients who were divided into 2 equivalent groups entered a retrospective investigation stage:

1A - 100 patients with two and more implantation failures of the thawed embryo in the anamnesis that continued treatment by the ART methods.

1B - 100 patients at whom clinical pregnancy occurred from the first attempt of transfer of the thawed embryos.

Patients of retrospective group 1B entered to the prospective group of this research (n=100). Depending on the chosen scheme of HRT endometrium preparation for transfer of the thawed embryo at a prospective investigation stage, they were divided into 2 groups also:

2A - 50 patients who during endometrium preparation for transfer of one thawed embryo received an oral form of estradiol valerate (EV) in a dose of 4 mg from 2nd day of menstrual period

2B - 50 patients who during endometrium preparation for transfer of one thawed embryo received an oral form of 17β-estradiol (17β–E) in a dose of 4 mg from 2nd day of menstrual period.

At a prospective stage, the preparation of endometrium for thawed embryo transfer was carried out with using of HRT and followed embryo transfer. The research came to the end for the 21st day after thawed embryo transfer and diagnosing of existence/absence of clinical pregnancy.

All patients were carried out the endometrium biopsy from 9 to 11 days of a menstrual period for assessment of endometrium receptor state as a result of HRT using manual vacuum aspirator of Ipas MVA Plus® Aspirator (USA). This menstrual period was previous a cycle with thawed embryo transfer.

Preservation of materials was carried out in 10.0% solution of formalinum with manufacture of paraffin blocks. Cuts of 4-5 microns were done and stained by hematoxylin and eosine [*Zhioua A. et al. 2012*].

The immunohistochemical research (IHC) was conducted by a standard technique with using of reactants of the DAKO company and mouse monoclonal antibodies to visualize an estrogen receptors – α-ER (clone 1D5), for estimation of proliferative activity – Ki-67 (clone of MIB-1) and for identification of plasmocytes – CD138 (clone MI15) [*Dabbs D. J., 2013*].

For the analysis of IHC reactions' results, the quantitative method used with calculation of the Histochemical score index (H-score) by the formula HS=1a +2b+3c where a – % of poorly painted cells, b – % of moderately painted cells; c – % of strongly painted cells; 1, 2, 3 – the intensity of coloring expressed in points.

The level of α-ER expression was estimated by result of H-score: 0–10 – the negative, 11–100 – low, 101–200 – moderate, 201 and more – high.

The morphometric study included measurement of percent of Ki-67 and amount of CD138 positive cells at magnification 400 in 10 poles with using Software DP-SOFT program and the subsequent statistical processing. Photography was carried out the OLYMPUS C 5050Z digital camera OLYMPUS CX41 fixed on a microscope with a video eyepiece of DCM-130E SCOPE.

Blood level of estradiol were determined on the 2-3rd, 6-7th, 10-11th, 14-16th days of a menstrual period and on day of an embryo transfer (the 19-21st day) by the enzymoimmunoassay (EIA) method on use of reagents' "DRG EIA Estradiol" (closed joint stock company "DRG of Tekhsistems, Russia) ranging from 60.0 *pmol/L* up to 1655 *pmol/L*. The normal indexes were defined in limits: a follicular phase — 68.0-1269.0 *pmol/L*, an ovulatory phase — 131.0-1655.0 *pmol/L*, a luteal phase — 91.0-861.0 *pmol/L*, according to the working standards and clinical recommendations at reproductive age women depending on menstrual period phase [*Vande Wiele R. L., et al., 1970*].

For statistical data processing, the following programs were used: IBM SPSS 19.0, Matlab 7. Paired comparisons for ranks serial this or the quantitative, but excellent on distribution from normal, were implemented by means of criterion U Mann Whitneys. For comparison of qualitative dichotomizing data, the criterion "Angular transformation of Fischer" with Yates amendment was used, at the same time the value of its statistics was designated as T. In all cases, the level of a statistical significance was designated as "p". Significant considered distinctions at the level $p<0.05$, highly significant — at the level $p<0.01$, very highly significant – at the level $p<0.001$ [*Altman D. G. et al., 2003*].

Results

The retrospective analysis data revealed an existence of clinical and anamnestic data similarity

and allowed to draw the conclusions on existence of the uniform mechanism of implantation failures in FET protocols at patients with RIF.

The analyzed groups 1A and 1B were comparable on an age (p=0.485), the social status (p=0.838) and body mass index (p=0.529). The analysis of the extragenital pathology showed its wider spread occurrence at patients of group 1B, in comparison with control group 1A.

In group 1B one chronic disease was diagnosed for 96.0% (96) patients at least, while in group 1A this index was 83.0% (83) (T=2.89, p=0.004). Only one disease of internals was diagnosed for 51.0% (51) of women at group 1A and 45,0% (45) at group 1B (T=0.71, p=0.479), two pathologies was diagnosed for 23.0% (23) in group 1A, in group 1B – for 34.0% (34) (T=1.57, p=0.118). Three and more extragenital diseases were diagnosed for 9.0% (9) and 17.0% (17) in groups 1A and 1B in accordance (T=1.48, p=0.140). Presence of two and more extragenital diseases showed statistically significant difference and it is risk factor of RIF with high probability (U=8781, p<0.001). There were no differences at extragenital pathology structure in the analyzed groups (p=0.850).

Gynecologic pathology occurred in 100.0% (100) women of groups 1A and 1B in the anamnesis. Most of patients of both groups had two gynecologic diseases in the anamnesis – 54.0% (54) and 68.0% (68) according to (T=1.79, p=0.075). Three and more gynecologic diseases had 21.0% (21) patients in group 1B and 12,0% (12) in group 1A (T=1.53, p=0.128). Only 11.0% (11) women had in the anamnesis one gynecologic pathology at group 1B while in group 1A this indicator was 35.0% (35) (T=3.99, p<0.001). The obtained data demonstrate that presence of two and more gynecologic pathologies have negative effect on the frequency of implantation rate at FET and can be regarded as risk factor of RIF (U=8696, p<0.001). The analysis of gynecologic diseases structure of the studied groups' patients revealed the statistically high significant (p<0.001) differences only for pelvic adhesive process – in group 1B this pathology was diagnosed by 1.84 times more often, in comparison with control group 1A.

Intrauterine pathology was diagnosed on average by 2.65 times more often in group 1B, than 1A (the relation of risks for groups 1A and 1B made 2.65, a confidential interval 1.63-4.30). Intrauterine pathology structure showed the statistically reliable difference in an endometrium hyperplasia (p<0.01), insufficiency of a luteal phase (p<0.01), an endometrium polyp (p<0.05) and a chronic endometritis (p<0.05) which were diagnosed more often in group 1B. The results of the carried-out statistical analysis allow to draw a conclusion that presence of any intrauterine pathology at the patient is risk factor of RIF (T=4.22, p<0.001).

Laparotomic pelvic surgeries in the anamnesis were carried out at the majority of patients in group 1B – 96.0% (96), in group 1A this indicator was 66.0% (66) (T=5.11, p<0.001). Statistical comparisons showed that the patients with any number of laparotomic pelvic surgeries (p<0.001) were by 1.45 times more often in group 1A, in comparison with group 1B (a confidential interval 1.26-1.68). The obtained data demonstrate that two and more laparotomic pelvic surgeries with high probability are risk factor of RIF (U=7168.0, p<0.001) probably because of morphological and functional disorders of pelvic organs integrity, their topics and blood supplies failures. Besides, of laparotomic pelvic surgeries, patients of the studied groups had a large number of intrauterine interventions in the anamnesis: hysteroscopies, endometrial currettage (diagnostic or connected with pregnancy) and biopsies.

At least one, an intrauterine intervention in both groups occurred at 100% (100) women in the anamnesis. In group 1B the patients prevailed who had 2 intrauterine interventions in the anamnesis – 39.0% (39). Most of patients at group 1A (84.0%, 84) were women who had only 1 intrauterine intervention in the anamnesis. Attracts attention, that 3 intrauterine interventions were at 33.0% (33) women in group 1B, 4 and more – at 15.0% (15) while in group 1A this number of interventions were not presented. The statistical analysis of the obtained data revealed that in group 1A two and more interventions were carried out to 6.08 times more often than in group 1B, therefore, existence of two and more intrauterine interventions is probable risk factor of RIF (U=6357, p<0.001) that is probably connected with microarchitecture disorders and morphological and functional integrity of endometrium.

The average duration of infertility was 6.86±0.34 years in group 1A and 9.9±0.35 in group 1B. In group 1A the most part patients suffered from infertility more than 10 years – 57.0% (57), from 5 to 9 years – 33.0% (33), at the same time, the group of patients suffered from infertility less than 5 years was only 10.0% (10). In group 1B the majority of women suffered from infertility from 5 to 9 years – 54.0% (54), and women suffered from infertility more than 10 years were presented to the smallest the number – 17 (17.0%). The statistical analysis revealed considerable influence of duration of infertility on the result of implantation – in group 1B the duration of infertility 10 years and more was observed at 3.35 times more often than in group 1A (a confidential interval 2.11-5.34) that allows to regard this criterion as one risk factors of RIF (U=7745, p<0.001).

The analysis of infertility structure also revealed a considerable difference in the analyzed groups. In group 1A the secondary infertility prevailed at 83.0% (83) while in group 1B – the primary (72.0%, 72) (T=8.15, p < 0.001). In structure of result of pregnancies at patients of group 1B with secondary infertility abortions prevailed – 91.6% (76) women had at least one abortion in the anamnesis. Results of statistical comparison of abortive pregnancies' shares of group 1A and 1B patients revealed that in group 1B abortions were observed 2.76 times more often, in comparison with group 1A (a confidential interval 1.92-3.97) (p<0.001). This study allows to consider the abortions are essential risk factors of the subsequent implantation failures (T=6.47, p<0.001).

The analysis of probable causes of infertility revealed the highest significant differences in distribution of quantity of probable causes of infertility in groups 1A and 1B. Patients with only one infertility cause at 46.0% (46.0) prevailed in group 1A (p<0.001) while the combination of several factors of infertility prevailed (T = 2.59, p = 0.010) in group 1B, the one factor of infertility diagnosed separately only at 21.0% (21). Presence of two and more probable causes of infertility reduces the probability of successful implantation by 1.72 times (a confidential interval 1.36-2.17) that allows to regard with high statistical reliability existence of two and more factors of infertility as risk factor of RIF.

Table 1

Risk factors of repeated implantation failures of the thawed embryo

Risk factor	Statistical importance
2 and more extragenital diseases	U = 8781, p <0.001
2 and more gynecological diseases in the anamnesis	U = 8696, p <0.001
Pathological changes in endomertrium	T = 4.22, p <0.001
2 and more pelvic organs surgeries in the anamnesis	U=7168.0, p <0.001
2 and more intrauterine interventions in the anamnesis	U=6357, p <0.001
Duration of infertility is more than 10 years	U=7745, p <0.001
2 and more factors of infertility	T = 3.65, p <0.001
Presence of 1 and more abortive pregnancies	T=6.47, p <0.001

Summarizing the previously mentioned, the presence of RIF risk factors of thawed embryo at a patient confirms reproductive system pathology, but their absence can be regarded as one of the factors of a successful outcome of the ART programs (Table 1).

The assessment of α-ER expression level in endometrium and a marker of proliferative activity of endometrium Ki-67 were carried out at a prospective stage and revealed a number of regularities in aspect from interrelation with the result of the thawed embryo implantation. The more expressed expression α-ER was noted in group 2B (207.2±1.13 points) in comparison with group 2A (123.6±0.69) (Figure 1, 2).

The similar situation developed in relation to an expression of cell proliferative activity marker Ki-67 both in glands and stroma: the percent of positively painted cells was 22.8±0.35 in glands (a weak expression of Ki-67) and 7.7±0.18 in stroma at patients of group 2A while these indicators were respectively 37.2±0.41 and 21.1±0.49 at patients of group 2B (Figure 3, 4).

Attracts attention that the clinical pregnancy rate is statistically authentically differed in the analyzed groups and was higher in group of patients 2B (Table 2).

Table 2

The immunohistochemistry results of endometrial biopsies at patients of the prospective group, abs.

Group of Patients (n - amount of patients)	α-estrogen receptors' expression M±m	Ki-67 expression		Clinical pregnancy rate abs., (%)
		gland epithelium M±m	stroma M±m	
2 A (n=50)	123.6±0.69	22.8±0.35	7.7±0.18	6 (12.0 %)
2 B (n=50)	207.2±1.13	37.2±0.41	21.1±0.49	16 (32.0 %)
The value of the criterion and the statistical significance of the differences	t=63.10 p <0.001	t=26.70 p <0.001	t=25.50 p <0.001	

Note: *M – mean value, m – standart deviation*

The carried-out analysis of estradiol blood level influence on the frozen embryo implantation result and its correlation with expression level in endometrium of α-ER and Ki-67 marker with the subsequent paired statistical comparison did not reveal reliable differences in the estradiol blood level at patients of the analyzed groups in any of menstrual period days (Table 3).

The obtained data demonstrates that the estradiol blood level does not correlate with the level of α-estrogen receptors in endometrium and cannot be used as a predictive factor of implantation result.

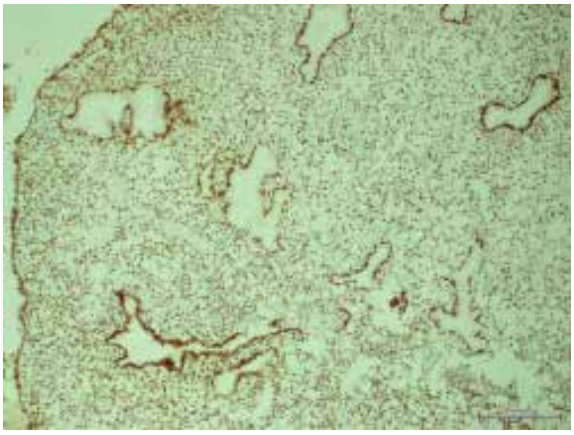

Figure 1. *Expressed expression of α-ER in an endometrial biopsy at a patient of group 2B. IHC. The increase is 100x.*

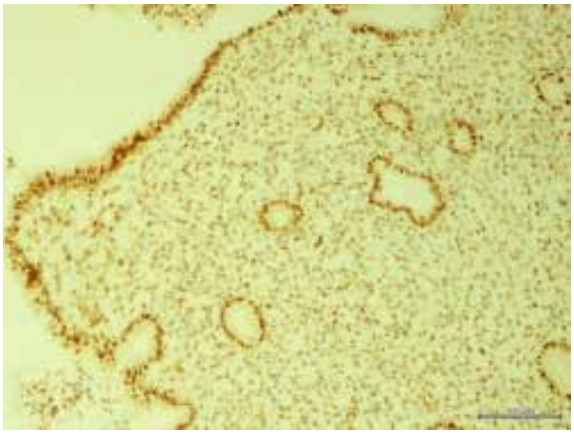

Figure 2. *Moderate expression of α-ER in an endometrial biopsy at a patient of group 2A. IHC. The increase is 100x.*

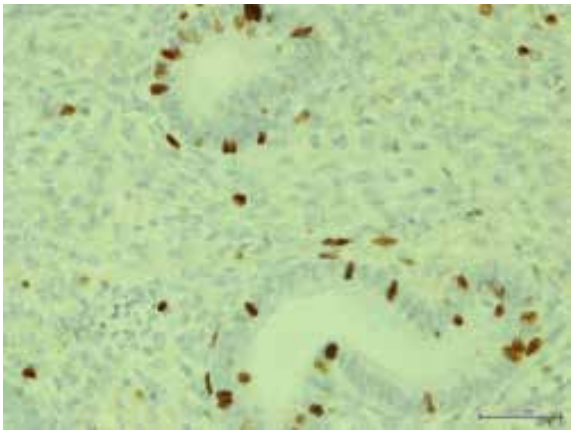

Figure 3. *Slightly expressed expression of Ki-67 in the glands and single positively stained stroma cells in the endometrial biopsy at the patient of group 1. IHC. The increase is 400x.*

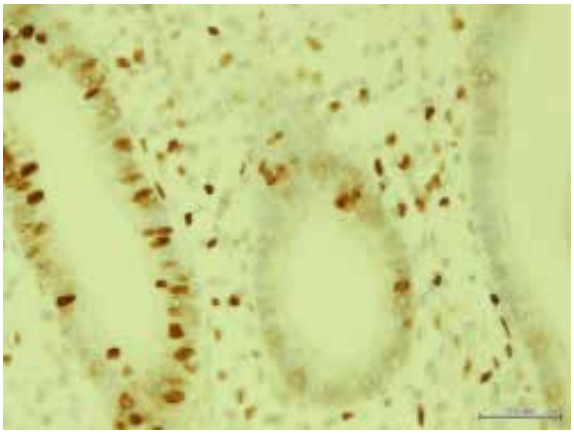

Figure 4. *Moderately expressed expression of Ki-67 in the epithelium of the glands and stroma of the endometrial biopsy at the patient of group 2. IHC. The increase is 400x.*

Discussion

The attempts of possible risk factors' identification of repeated implantation failures in ART programs were made in a number of researches. So, Vartanyan E. V.'s studies showed the negative impact of a late reproductive age women on IVF outcomes, the degenerative changes in oocytes against the background of the progressing reduction of an ovarian reserve [*Vartanyan E. V., 2012*] that is also confirmed researches of other authors [*Shmidt, L., 2007; Dominguez F. et al., 2010*]. In other researches, the classical autoimmune diseases (including an autoimmune thyroiditis) presented like the probable factor of repeated implantation failures because of violation of immune mechanisms of implantation [*Bellver J. et al., 2008*]. Other authors found that the one of probable causes of repeated implantation failures was chronic inflammatory diseases of the reproductive sphere in view of local inflammatory changes in endometrium and system violations among which the hypothalamic-hypophyseal-ovarian axis dysfunction as a result of a pathological afferentation, leading to decrease in secretion of progesterone and estrogen and depressing of the immune system which is shown in decrease of secretion of interferons and decrease of the activity of NK cells [*Cicinelli E. et al., 2008*]. However, despite the unconditional significance and the scientific interest of these researches, their results are extremely separated and fragmentary and did not give the complete idea about repeated implantation failures' risk factors.

Table 3

Evaluation of estradiol blood level in two groups of patients at the prospective study stage

Day of the menstrual period	Estradiol blood level, picomol per liter		Significance of differences
	Group 2А M±m	Group 2Б M±m	
2–3	76.14±0.06	71.43±0.13	p = 0.672
6–7	325.94±0.74	323.87±1.1	p = 0.345
10–11	661.18±0.70	635.25±0.93	p = 0.631
14–16	1476.53±0.96	1465.78±0.87	p = 0.795
19–21	793.21±0.67	778.33±0.79	p = 0.573

Note: *M – mean value, m – standart deviation*

It is confirmed all previously noted unfavorable factors. And statistically approved predictively favorable factors of clinical pregnancy in FET cycles at patients with the RIF are:

- two and more gynecologic diseases in the anamnesis;
- two and more extragenital diseases;
- two and more pelvic organs surgeries in the anamnesis;
- two and more intrauterine interventions in the anamnesis;
- two and more factors of infertility
- absence of pathologic morphological changes at endometrium after miscarriages in the anamnesis;
- duration of infertility is less than 10 years.

For estimation of functional activity of endometrium as key factor of effectiveness ART's programs and as markers of unsuccessful implantations' development by some researches was suggested integrins, for example the decreasing of αVβ3-integrin level was revealed in frequent unsuccessful implantations at women with endometriosis [*Jiang L, Xu W, 2009*]. And the decreasing of mucin-1 (MUC–1) level was diagnosed in an middle luteal phase at patients with repeated miscarriages as well as the increasing of short modification of MUC-1 allele frequencies at patients with repeated implantation failures [*Horne A. W. et al., 2001; Hannan N. J. et al., 2010*]. However, despite prospects of this direction of a research, the specified markers of unsuccessful implantation were not investigated at patients with repeated unsuccessful protocols while in our research the analysis of degree of alpha estrogen receptors' expression revealed their direct influence on thawed embryo transfers' outcomes and clinical pregnancy rate. But in our investigation it was established the more expressed expression α-ER of endometrium and a marker of proliferative activity of endometrium (Ki-67) reduces risk of failure implantation by 1.33 times at patients with repeated failure implantations of thawed embryo (a confidential interval 1.05-1.67) and, respectively, increases the probability of clinical pregnancy rate by 21.5% (a confidential interval of 4.5-37.3%) that the expressed expression of these receptors in endometrium may be seen like a favorable factor in FET protocols.

CONCLUSION

The identified risk factors of repeated implantation failures at the frozen embryo transfer have high statistical reliability and can successfully be used for the forecasting of successful implantation. The expressed expression of endometrium α-estrogen receptors and a marker of proliferative activity of endometrium (Ki-67) identified on 9-11 days of menstrual period, is associated with increasing of clinical pregnancy rate in FET protocols Estradiol blood level does not correlate with the level of α-estrogen receptors expression and Ki-67 marker in endometrium and is not associated with the clinical pregnancy rate.

REFERENCES

1. *Altman DG, Machin D, Bryant TN, Gardner MJ.* Statistics with confidence. Confidence intervals and statistical guidelines. Bristol: BMJ Books, 2003.
2. *Bellver J, Soares S, Alvarez C., et al.* The role of thrombophilia and thyroid autoimmunity in unexplained infertility, implantation failure and recurrent spontaneous abortion. Human Reproduction. 2008; 23(2): 278-284.
3. *Cicinelli E, De Ziegler D, Nicoletti R., et al.* Chronic endometritis: correlation among hysteroscopic, histologic, and bacteriologic findings in a prospective trial with 2190 consecutive office hysteroscopies. Fertility and sterility. 2008; 89(3): 677–684.
4. *Crum CP, Hornstein MD, Steward EA.* Evolution of cyclic endometrium and, benign endometrial disorders. Diagnostic Gynecologic and Obstetric Pathology. Philadelphia: Saunders. 2006; 441-488.
5. *Dabbs DJ.* Diagnostic Immunohistochemistry: Theranostic and Genomic Applications. Elsevier, 4-th Edition. 2013: 960.
6. *Devyatova EA, Tsaturova KA, Vartanyan EV.* Predicting of successful implantation at IVF cycles. Gynecological Endocrinology. 2016; 32(1): 27-29.
7. *Dominguez F, Gadea B, Mercader Esteban A., et al.* Embryologic outcome and secretome profile of implanted blastocysts obtained after coculture in human endometrial epithelial cells versus the sequential system. Fertility and sterility. 2010; 93(3): 774-782.
8. *Dzhamaludinova AF, Gonyan MM.* [Reproductive health of the Russian population] [Published in Russian]. Young scientist (Kazan). 2017; 14(2): 10-13.
9. *Gajdukov SN, Boyarskij YuK, Pal'chenko NA.* [Modern view on the problem of receptivity and thin endometrium in art programs (literature review)] [Published in Russian]. Russian Journal of Human Reproduction. 2013; 4: 51-60.
10. *Hannan NJ, Paiva P, Dimitriadis E., et al.* Biology of Reproduction. 2010; 82(2): 235-245.
11. *Horne AW, White JO, Margara RA., et al.* MUC1: a genetic susceptibility to infertility? The Lancet. 2001; 357(9265): 1336-1337.
12. *Jiang L, Xu WM.* Expression of estrogen receptor, progesterone receptor and integrin-alphaV beta3 in endometrium of patients with luteinized unruptured follicle. National Medical Journal of China. 2009; 44: 929-932.
13. *Korsak VS.* [ART Register 2016] [Published in Russian]. Saint-Petersburg. 2018: 20-48.
14. *Mazur MT, Kurman RJ.* Diagnostic of endometrial biopsies and curreting: A practical approach. New York: Springer. 2005. 296p.
15. *Nastri CO, Ferriani RA, Rocha IA., et al.* Ovarian hyperstimulation syndrome: pathophysiology and prevention. Journal of Assisted Reproduction and Genetics. 2010; 27: 121-128.
16. *Nosenko EN, Saenko AI, Postolyuk IG.* [Receptor status of endometrium in infertile women with a history of unsuccessful attempts of assisted reproductive technologies] [Published in Russian] Tavricheskiy Mediko-Biologicheskiy Vestnik. 2013;16(2;2): 80-82.
17. *Pandey S, Shetty A, Hamilton M, Bhattacharya S, Maheshwari A.* Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a systematic review and meta-analysis. Hum Reprod Update. 2012; 18: 485-503.

18. *Paulson RJ.* Hormonal induction of endometrial receptivity. Fertility Sterility. 2011; 96(3): 530-535.

19. *Roque M, Valle M, Guimarães F, Sampaio M, Geber S.* Freeze-All Policy: Fresh vs Frozen-Thawed Embryo Transfer. Fertil Steril. 2015; 103: 1190-1193.

20. *Ruiz-Alonso M, Blesa D, Díaz-Gimeno P, Gómez E, Fernández-Sánchez M., et al.* The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. Fertil Steril. 2013; 100(3): 818-824.

21. *Schoolcraft WB, Gardner DK.* Blastocyst versus day 2 or 3 transfer. Semin Reprod Med. 2001; 19: 259-268.

22. *Shmidt L.* Effect of infertility insurance mandates on fertility. The European Journal of Health Economics. 2007; 26(3): 336-366.

23. *Simon A, Laufer N.* Assessment and treatment of repeated implantation failure (RIF). J Assist Reprod Genet. 2012; 29(11): 1227-1239.

24. *Tehraninejad ES, Kabodmehri R, Rashidi BH., et al.* Trans dermal estrogen (oestrogel) for endometrial preparation in freeze embryo transfer cycle: An RCT. Int J Reprod Biomed (Yazd). 2018; 16(1): 51-56.

25. *Vande Wiele RL, Bogumil J, Dyrenfurth I, Ferin M, Jewelewicz R., et al.* Mechanisms regulating the menstrual cycle in women. Recent Prog Horm Res. 1970; 26: 63-103.

26. *Vartanyan EV.* [The possibility of overcoming repeated failures in the procedures of assisted reproductive technologies] [Published in Russian]. Vestnik uralskoi meditsinskoi akademicheskoi nauki. 2013; 3(45): 56-62.

27. *Zhioua A, Elloumi H, Fourati S., et al.* [Morphometric analysis of the human endometrium during the implantation window. Light and transmission electron microscopy study] [Published in French]. J Gynecol Obstet Biol Reprod (Paris). 2012; 41(3): 235-242.