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UNEXPLAINED INFERTILITY: CLINICAL CHARACTERISTICS OF COUPLES AND EMBRYOLOGICAL FEATURES OF IN VITRO FERTILIZATION PROGRAMS

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ABSTRACT

Introduction: The specialists refer unexplained infertility to the so-called diagnosis of exclusion due to the fact that in the process of medical examination of married couples the causes of infertility cannot be established.

Material and methods: The clinical characteristics and embryological features of in vitro fertilization programs of couples with unexplained infertility versus the patients with tuboperitoneal infertility were analyzed retrospectively and prospectively.

The study group comprised 93 women, who underwent 108 in vitro fertilization programs, and the control group consisted of 45 patients, who underwent 49 in vitro fertilization programs.

Results: Significant differences ($p < 0.05$) were found between the groups in anamnestic, clinical, laboratory, and instrumental characteristics. The ovarian stimulation protocols were comparable between the groups of patients. The blastulation rate was considered to be the endpoint in in vitro fertilization programs, and it was significantly lower in the group of women with unexplained infertility (45.53%). In-depth analysis of the embryonic stage of in vitro fertilization programs showed, that low blastulation rate in unexplained infertility was mainly due to the fact that embryos stopped developing about three days after they were cultured. At the same time the morphological assessment showed that the quality of blastocysts was higher in the group of unexplained infertility (66.7%) compared to the group of tuboperitoneal factor of infertility (45.8%). Preimplantation genetic testing for aneuploidy showed similar frequency of detection of euploid embryos (41.7% and 40.0%, respectively).

Conclusion: A “clinical portrait” of women with unexplained infertility was described. The low blastulation rate was noted in in vitro fertilization programs for women with unexplained infertility. Given the identified impairments of early embryonic development in unexplained infertility, it is appropriate to recommend the patients to undergo early use of in vitro fertilization with good-quality embryo transfer (>3 , AA, AB, BA according to Gardner grading system) on day 5-6 of culture without long-lasting preliminary examination and empirical treatment.

KEYWORDS: unexplained infertility, tuboperitoneal factor of infertility, oocyte factor, embryonic development, in vitro fertilization, assisted reproductive technologies.

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INTRODUCTION

The diagnosis of unexplained infertility is established in couples whose infertility cannot be defined in the process of standard medical examination: regular, ovulatory menstrual cycle; absence of pathological changes in endometrium; normal semen parameters, patent fallopian tubes, absence of pathology by the results of laparoscopy and hysteroscopy, so it is categorized by specialists as a diagnosis of exclusion [Breitkopf D et al., 2019; Buckett W, Sierra S, 2019; Penzias A et al., 2020]. According to different sources, the frequency of unexplained infertility significantly varies and reaches 10-30% among infertile couples, and 10-17% among female infertility [Berek J et al., 2019; Cariati F et al., 2019; Wang R et al., 2019].

Unexplained infertility continues to remain an enigma for the doctors and researchers dictating both necessity for further research of the reproductive system of those patients, who have no obvious causes of infertility, and the improvement of clinical tactics aimed at the effective realization of reproductive function.

According to the published data, there are few studies in the world, which are devoted to unexplained infertility, and their level of evidence is considered low. International consensus development study established top ten priorities for future infertility research, including unexplained infertility [Duffy J et al., 2020]. Some studies suggest that additional examination of couples with unexplained infertility, does not increase the incidence rate of the onset of pregnancy [NICE, 2017]. It is recommended to take into account the age, duration of infertility, individual characteristics of patients when drawing up a plan for the diagnosis and treatment of unexplained infertility [Nandi A et al., 2015; Gunn D, Bates G, 2016]. The specialists assess differently ovarian reserve status in women among married couples with unexplained infertility. Some of them point out to low ovarian reserve parameters compared to the patients of similar age, who have tuboperitoneal factor of infertility, and others – to the absence of these differences [Yücel B et al., 2018; Bosch E et al., 2021; Kirakosyan E et al., 2022b]. The disagreement in the definition of the term “unexplained infertility” may be partly due to late admission of patients to in vitro fertilization clinics, prolonged watch-and-wait strategy

and empirical treatment, which are used because the cause of infertility is not clear [Abrahami N et al., 2019; Buckett W, Sierra S, 2019]. At the same time, undoubtedly that the most important factor limiting the effectiveness of in vitro fertilization programs is a woman's age [Yücel et al., 2018; Siristatidis C et al., 2020].

Currently, there are no convincing data on the differences in live birth rates in expectant management and in the use of assisted reproductive technologies – in vitro fertilization without or with intracytoplasmic sperm injection into the oocyte [Siristatidis C et al., 2020]; as well as no markers suggesting the advantages of the use of one or another method of assisted reproductive technologies for the patient [Tjon-Kon-Fat R et al., 2017].

The absence of obvious anatomical abnormalities and abnormal reproductive system physiology in partners – married couples, enabled the researchers to analyze the embryological parameters in in vitro fertilization programs in patients with unexplained infertility. However, there are practically no thorough researches that provide objective information about the quality of oocytes, their ability to fertilize, the features of embryogenesis in patients diagnosed with unexplained infertility. This fact determined the necessity to carry out this study.

The **purpose** of the study was to analyze the clinical characteristics and embryological parameters in in vitro fertilization programs for women with unexplained infertility versus the patients with tuboperitoneal factor of infertility.

THE STUDY DESCRIPTION

Research method was continuum, goal-oriented and focused on patients seeking healthcare in Scientific and Clinical Department of Assisted Reproductive Technologies named after F. Paulsen, the National Medical Research Center for Obstetrics, Gynecology and Perinatology named after Academician V.I. Kulakov of the Ministry of Health of the Russian Federation; the period for prospective study of unexplained infertility was from January 01, 2021 to December 13, 2021, retrospective study of unexplained infertility from – January 1, 2019 to August 11, 2021, tuboperitoneal factor of infertility from January 1, 2019 to October 22, 2021.

The type of the study was observational, analytical, cohort, mixed (retrospective-prospective).

At the preparatory stage of the study the issues of diagnostics, treatment of patients with unexplained infertility were defined and publications on the relevant problem were reviewed [Kirakosyan E et al., 2021]. The aim and the goal of research were formulated and the working hypothesis was developed – the assumption that the patients with unexplained infertility have certain clinical characteristics and embryological parameters in in vitro fertilization treatment programs.

The stage I was scheduling the program and statistical analysis plan.

The program included:

1. determination of the observation unit (Materials and methods. Inclusion criteria) and scheduling the program of material collection (sequential reporting of considered characteristics-questions, and necessity to get answers to the questionnaire);
2. scheduling the program of material development (drawing up the layouts of statistical tables based on the considered characteristics-questions and the answers received to unexplained infertility, registry of tuboperitoneal factor of infertility (the control group), embryology of unexplained infertility, embryology of tuboperitoneal factor of infertility (the control group);
3. scheduling the program for the analysis of the collected material (the list of statistical methods, which were necessary to identify the patterns of the phenomenon under the study (Materials and methods. Statistical data processing).

Research plan:

1. the subject of research: 93 women with unexplained infertility in in vitro fertilization programs;
2. statistical sample size: 45 women prospectively, 48 women with unexplained infertility and 45 women with tuboperitoneal factor of infertility retrospectively. The samples are qualitatively and quantitatively representing the general populations, respectively, and are comparable by the number of participants.
3. the type of the study: observational, analytical, cohort, mixed (retrospective-prospective); material collection method and the terms of the study: goal-oriented (according to criteria established for this study), continuous (taking into account all cases), current (registration of

detected cases); focused on patients seeking healthcare in Scientific and Clinical Department of Assisted Reproductive Technologies named after F. Paulsen, the National Medical Research Center for Obstetrics, Gynecology and Perinatology named after Academician V.I. Kulakov of the Ministry of Health of the Russian Federation; the period under study: unexplained infertility prospectively from January 01, 2021 to December 13, 2021, unexplained infertility retrospectively from – January 1, 2019 to August 11, 2021, and tuboperitoneal factor of infertility from January 1, 2019 to October 22, 2021.

At stage II, material was collected according to the scheduled program and research plan.

At stage III, the obtained data were processed (verification of complete collection of research material, grouping, encryption, summary of data for statistical tables, calculation of statistical parameters and statistical material processing).

At stage IV, the obtained results were analyzed, the conclusions and suggestions were made.

MATERIALS AND METHODS

The clinical characteristics of 1191 couples with infertility were analyzed retrospectively and prospectively, of which 93 women from couples with unexplained infertility were included in the study group and 45 women with tuboperitoneal factor of infertility were included in the control group. Comparative analysis of clinical characteristic and major parameters in in vitro fertilization programs was performed in 108 programs for women with unexplained infertility and 49 programs in patients with tuboperitoneal factor of infertility, who underwent fertility treatment in in vitro fertilization Departments of the National Medical Research Center for Obstetrics, Gynecology and Perinatology named after Academician V.I. Kulakov of the Ministry of Health of the Russian Federation in the period from 2019 to 2021. The ovarian stimulation protocols were comparable between the groups of patients. Severe deviations in spermogram parameters and possible infertility factors in the partners were excluded (Fig. 1).

The incidence of unexplained infertility in the study group was from 6.07% retrospectively to 11.25% prospectively.

Tuboperitoneal factor of infertility was diagnosed by hysterosalpingography and/or laparoscopy.

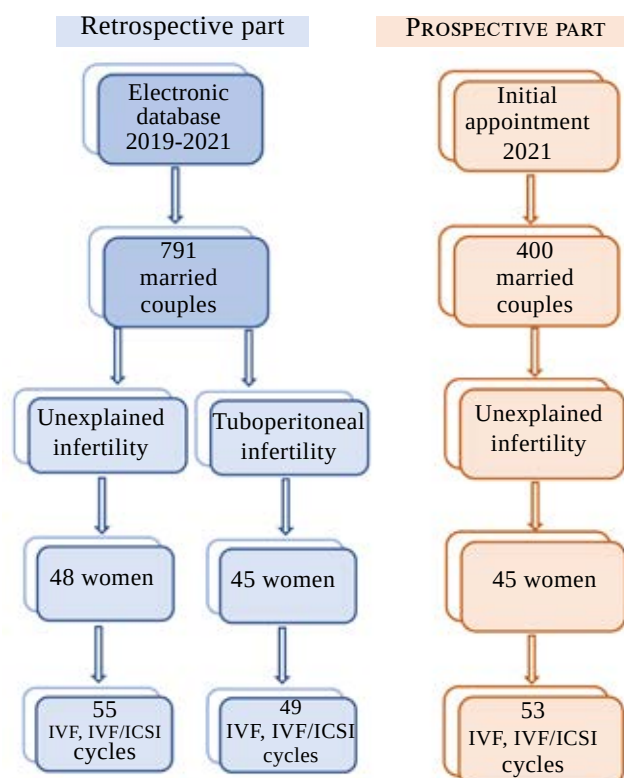


FIGURE 1. Design of the study, where IVF is in vitro fertilization and ICSI - intracytoplasmic sperm injection

Unexplained infertility as diagnosis of exclusion was established in:

1. occurrence of ovulation confirmed by regular menstrual cycle length of 23-35 days, ovulation test, measurement of progesterone level in the blood during luteal phase of the menstrual cycle; visualization of the corpus luteum by ultrasound monitoring and/or laparoscopy in the luteal phase of the menstrual cycle;
2. normal uterine cavity according to ultrasound examination and/or hysteroscopy and tubal patency assessment by hysterosalpingography and/or laparoscopy;
3. compliance of spermogram results with reference values of the World Health Organization [WHO, 2010].

Inclusion criteria for unexplained infertility:

- age of women ≤ 35 years at the time of diagnosed infertility;
 - duration of infertility ≥ 3 years;
 - no obvious reasons for infertility;
 - male partner with normozoospermia;
 - normal female (46, XX) and male (46, XY) karyotypes.
- Patient evaluation included:

- anamnestic data collection (questionnaire);
- examination of patients according to the requirements for undergoing in vitro fertilization programs.

The patients underwent treatment in Scientific and Clinical Department of Assisted Reproductive Technologies named after F. Paulsen, the National Medical Research Center for Obstetrics, Gynecology and Perinatology named after Academician V.I. Kulakov of the Ministry of Health of the Russian Federation based on standard protocols routinely used in the in vitro fertilization Departments, which are in compliance with the international and European criteria of quality. The ovarian stimulation was performed with gonadotropin-releasing hormone antagonist protocol, and less commonly gonadotropin-releasing hormone agonist long protocol. The starting daily dose of gonadotropins was selected individually depending on the body mass index, patient's age, the level of anti-Müllerian hormone and antral follicle count and averaged 225 IU.

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All manipulations, cultivation conditions, and work of medical personnel was performed in the same manner as it has been done for more than 5 years of functioning of the Department and the standards. In vitro fertilization/intracytoplasmic sperm injection was performed according to indications: failed or low frequency ($<20\%$) of fertilization in the previous in vitro fertilization attempt, multiple in vitro fertilization attempts in medical history. Cultivation was carried out in single step culture medium in conditions of low oxygen levels (5%

O₂, 6% CO₂, 89% N₂). No more than two embryos were transferred into the uterus. The average number of transferred embryos in unexplained infertility group was 1.0 embryo per woman, in tuboperitoneal factor of infertility group – 1.2 embryo per woman.

The Gardner grading system was used for morphological assessment of the quality of blastocysts. According to the Istanbul consensus

workshop on embryo assessment (2011) and the Vienna consensus meeting (2017), this grading system is currently a major embryological criterion for embryo quality because of its prognostic significance [Gardner D, Schoolcraft W, 1999 a; b; Balaban B et al., 2011; Apter S et al., 2017]. Evaluation of blastocysts was based on the analysis of trophoblast cells, inner cell mass and the cavity size. The degree of blastocyst expansion or formation of the blastocyst cavity was assessed using grading scale (1-6 grades). The inner cell mass and trophoblast were graded on a scale of ABC. This scoring system implies an unambiguous division of the blastocyst into trophoblast and inner cell mass and their independent assessment, which are impossible for stage 1, and may be difficult for stage 2.

At the first stage of the study, anamnestic, clinical, laboratory, and instrumental parameters were evaluated in patients with unexplained infertility and tuboperitoneal factor of infertility (Table 2).

Based on these findings, the second stage involved analyzing the embryonic stage of in vitro fertilization programs – both in vitro fertilization and intracytoplasmic sperm injection, in patients with unexplained infertility compared to those with tuboperitoneal factor of infertility (Table 3).

For this purpose, the following ratios were also determined: mature oocytes (%), bipronuclear zygotes (%), and blastulation rate (%) respectively.

TABLE 1.

Parameters of in vitro fertilization programs in the groups of patients with unexplained infertility and tuboperitoneal factor of infertility

Feature	Unexplained infertility $n_1=93, n_2=108$	Tuboperitoneal factor of infertility $n_1=45, n_2=49$
Protocol with GnRH antagonists	89%	84%
Dose/day (IU)	225	225
Dose/course (IU)	2475	2685
Days of stimulation	11	11
Number of pre-ovulatory follicles	12.3	11.1
Number of aspirated oocytes	10.8	9.0
Number of mature oocytes	9.6	7.3
Number of zygotes	7.6	5.8

NOTES: n_1 – number of patients, n_2 – number of in vitro fertilization programs, $p \geq 0.05$ – the absence of statistically significant differences between the groups

TABLE 2.

Mean values of the clinical characteristics in the groups of patients with unexplained infertility and tuboperitoneal factor of infertility

Feature	Unexplained infertility ($n_1=93, n_2=108$)	Tuboperitoneal factor of infertility ($n_1=45, n_2=49$)	t	p
Age of women (years)	33 (0.8)	33 (0.7)	0	
Partner's age (years)	34 (1.2)	36 (2.3)	2.06*	≥ 2.01 <0.05
Body weight of women (kg)	62.6 (4.1)	69.1 (7.9)	1.93	
Height of women (cm)	165.0 (1.7)	167.0 (3.0)	1.95	
BMI (kg/cm ²)	22.9 (1.4)	24.8 (3.0)	4.03*	≥ 3.52 <0.001
Menarche age (years)	13.3 (0.3)	13.0 (0.3)	5.40*	≥ 3.52 <0.001
Menstrual cycle length (days)	28.0 (0.6)	31.5 (4.2)	1.67	
Duration of infertility (years)	5.7 (0.9)	4.8 (1.0)	6.43*	≥ 3.52 <0.001

NOTES: n_1 – number of patients, n_2 – number of in vitro fertilization programs, t – t criterion, *- the presence of statistically significant differences between the groups

$$\text{Mature oocytes} = \frac{\text{number of mature oocytes}}{\text{number of aspirated oocytes}} \times 100$$

$$\text{Bipronuclear zygotes} = \frac{\text{number of zygotes}}{\text{number of mature oocytes}} \times 100$$

$$\text{Blastulation rate} = \frac{\text{number of blastocysts}}{\text{number of zygotes}} \times 100$$

Statistical analysis: Clinical and embryological data base were created using Microsoft Excel software program and were used for storage and preliminary data processing. Statistical data processing was performed using absolute, relative and mean values, criteria to measure diversity of variation series, standardization method, parametric methods for assessment of research reliability: to measure representativeness errors, assess statistical significance of the difference in the results of the study (t criterion), calculate confidence interval for mean and relative values [Kucherenko V, 2011]. The Student's t-test was used to compare quantitative variables, and Student's t-table was used for the critical values. The differences were considered statistically significant at $p < 0.05$. The blastulation rate was considered to be the endpoint in in vitro fertilization treatment programs.

Ethical approval: This study was approved by the Academic Council of the Sechenov University (Order No. 4070/OP-32, 30.09.2020), the Local Ethics Committee of the Sechenov University (Protocol No. 33-20, 25.11.2020), the Biomedical Research Ethics Commission of the National Med-

ical Research Center for Obstetrics, Gynecology and Perinatology named after Academician V.I. Kulakov (Protocol No. 11, 12.11.2020). The study was performed in accordance with the Federal Law of the Russian Federation of 27.07.2006 No. 152-FZ (as amended on 29.07.2017) "On Personal Data", with the Federal Law of the Russian Federation of 21.11.2011 No. 323-FZ "On the basis of health care in the Russian Federation" (Article 13 "Respect for medical confidentiality"), the Order of the Ministry of Health of the Russian Federation of 31.07.2020 No. 803n "On the procedure for the use of assisted reproductive technologies, contraindications and restrictions on their use", the clinical guidelines of the Ministry of Health of the Russian Federation "Female infertility", the provisions of the Declaration of Helsinki for medical research involving human subjects, the International ethical guidelines for biomedical research involving human subjects of the Council for International Organizations of Medical Sciences. Patients signed informed consent to participate in the study, including the use of their data in publications.

RESULTS

At stage I of the study, the anamnestic, clinical, laboratory and instrumental parameters in patients with unexplained infertility and tuboperitoneal factor of infertility were evaluated (Table 2)

At stage I of the study, the anamnestic, clinical, laboratory and instrumental parameters in patients with unexplained infertility and tuboperitoneal factor of infertility were evaluated.

In unexplained infertility group, the number of women with cancer burden was statistically higher – 47% or with diabetes mellitus – 44% in hereditary anamnesis versus tuboperitoneal factor of infertility group (24% and 14%, respectively).

Evaluation of somatic health of women in the study groups showed that significantly more often thyroid disorders were in unexplained infertility group – in 31% of women and diseases of digestive system were in 29% of women. In tuboperitoneal factor of infertility group significantly more often were gynecologi-

Mean values of embryological parameters in vitro fertilization programs in the groups of patients with unexplained infertility and tuboperitoneal factor of infertility

Feature	Unexplained infertility ($n_1=93$, $n_2=108$)	Tuboperitoneal factor of infertility ($n_1=45$, $n_2=49$)	t
Mature oocytes (%)	73.8 (4.4)	75.1 (8.0)	0.4
Bipronuclear zygotes (%)	76.5 (4.6)	78.1 (7.3)	0.6
fertilization method - IVF (%)	19.7	41.7	
fertilization method - ICSI (%)	80.3	58.3	
Blastulation rate (%)	45.5 (6.8)	57.3 (11.4)	2.56*

Notes: n_1 – number of patients, n_2 – number of in vitro fertilization programs, IVF – in vitro fertilization, ICSI – intracytoplasmic sperm injection, * $t \geq 2.45$ ($p < 0.05$) – the presence of statistically significant differences between the groups

TABLE 3.

cal diseases – in 66% of patients (endometriosis, adenomyosis was in 13%, mastopathy in 11%, fibroids in 9%, chronic endometritis in 9%, chronic salpingo-oophoritis in 7%, polycystic ovary syndrome in 4%, other diseases in 13% of patients), including pelvic inflammatory disease in 38% of women, which were unspecific for unexplained infertility group (11% and 13%, respectively).

Statistically, the number of women, who underwent laparoscopy – 94%, hysteroscopy – 88%, endometrial biopsy 83%, the results of which showed concomitant changes – 50%, other interventions – 82%, including laparotomy – 64%, restoration of tubal patency – 33% was significantly higher in tuboperitoneal factor of infertility group (47%, 58%, 56%, 27%, 24%, 2% and 7%, respectively) and this was specific for pathogenesis of tuboperitoneal factor of infertility.

According to the anamnestic data, the presence of ectopic pregnancies, pelvic inflammatory disease, gynecological diseases, interventions for the diagnosis and treatment of pelvic organs and the abdominal cavity were in 94% of patients with tuboperitoneal factor of infertility, and only in 58% of women with unexplained infertility, who underwent mainly diagnostic interventions.

The differences between unexplained infertility group and tuboperitoneal factor of infertility group with regard to pregnancies that ended with births in anamnesis in 11% and 21% of women, respectively; and with regard to pregnancies with adverse outcomes in anamnesis in 44% and 54% of women, respectively.

Statistically, the average duration of infertility was significantly longer in unexplained infertility group – 5.65 years versus 4.75 years in tuboperitoneal factor of infertility group. At the same time, the number of women with duration of infertility more than 6 years was significantly higher in unexplained infertility group – 43% versus 29% in tuboperitoneal factor of infertility group. In unexplained infertility group the number of women who used contraception before pregnancy was 76% versus 19% in the other group. Presumably, this was associated with patients' awareness about low probability of spontaneous pregnancy in cases of tuboperitoneal factor of infertility.

Statistically, the number of women with previously established diagnosis of infertility factor was

significantly higher in tuboperitoneal factor of infertility group – 100% versus 69% in unexplained infertility group. The number of women in tuboperitoneal factor of infertility group, who underwent assisted reproductive technologies in anamnesis with the purpose to conceive, was significantly higher – 89% versus 67% in unexplained infertility group. In unexplained infertility group, 27% of patients had intra-uterine insemination in anamnesis: in 25% of women it failed, 2% of women had miscarriage. Moreover, the average time period before seeking healthcare in the in vitro fertilization clinic in this subgroup was 6.33 years. The number of women with failed in vitro fertilization attempts in medical history was significantly high in tuboperitoneal factor of infertility group – 54% versus 38% in unexplained infertility group. Statistically significant differences between the groups in the outcomes of in vitro fertilization programs in anamnesis were not found: birth rates were 4.4% and 5.4%, miscarriage – 2.2% and 5.4%, missed miscarriage – 7% and 5.4%, biochemical pregnancy – 4.4% and 8%, respectively. The number of women with one in vitro fertilization attempt in anamnesis was significantly high in tuboperitoneal factor of infertility group – 39%, and in unexplained infertility group with two UVF attempts in anamnesis – 36 %. The differences between the groups with 3 or more in vitro fertilization attempts in anamnesis were not found.

The obtained data can be explained by the fact, that patients with unexplained infertility do not undergo medical screening over the course of many years, due to this the cause of infertility remains unexplained, and the attempts to conceive or “recover” fail. This is the reason why the patients seek in vitro fertilization too late. In patients with tuboperitoneal factor of infertility, the cause of infertility is obvious, and in vitro fertilization is the only possible way to achieve pregnancy for them, so the patients timely seek appropriate medical care.

Indicators of ovarian reserve status – the level of anti-Müllerian hormone not less than 1.2 ng/mL and antral follicle count not less than 5 assessed by ultrasound imaging in patients in the follicular phase, corresponded with the values regulating the in vitro fertilization program according to the clinical recommendations. Statistically significant differences between the mean values of anti-Müllerian hormone in the groups (2.44 ng/mL in unexplained infertility

group and 2.34 ng/mL in tuboperitoneal factor of infertility group). According to US assessment in the follicular phase, the number of women with multifollicular ovaries was significantly high (37%) in tuboperitoneal factor of infertility group, and a high number of women with 8-12 antral follicles (31%) was in unexplained infertility group.

Thus, a “clinical portrait” of the patient with unexplained infertility was described: a woman aged 33 years (the partner’s age 34 years), with body mass 62.56 kg, height 164.95 cm, normal body mass index 22.85 kg/cm², age at menarche 13.31 years, duration of the menstrual cycle 28.04 days, period length 4.69 days, moderate period pain score of 5.17 points, duration of infertility 5.65 years, cancer or diabetes mellitus in hereditary anamnesis in the half of cases, with thyroid disorders or diseases of digestive system were the one third of cases, absence of infectious or non-infectious gynecological diseases, no pelvic surgery, births in anamnesis in one tenth of cases, adverse pregnancy outcomes in anamnesis in half of cases, previously diagnosed infertility and two in vitro fertilization attempts in two thirds of cases, birth rate as a result of in vitro fertilization 4.4-7.4%, normal ovarian reserve according anti-Müllerian hormone test – 2.44 ng/mL and antral follicle count – 8-12 in the follicular phase.

At the second stage of the study, it was analyzed the embryonic stage of in vitro fertilization programs (in vitro fertilization and intracytoplasmic sperm injection) in patients with unexplained infertility compared to patients with tuboperitoneal factor of infertility (Table 3).

According to obtained mean values, the differences in the number of obtained mature oocytes between the groups were not statistically significant. Despite the fact that intracytoplasmic sperm injection was used as a fertilization technique most often in unexplained infertility group (80.3%) compared to tuboperitoneal factor of infertility group (58.3%), there was no statistically significant difference between the average rates of fertilization in the groups. Most important is that statistically the average blastulation rate in in vitro fertilization programs was significantly low in unexplained infertility group (Fig. 2).

Reduction of blastulation rate in unexplained infertility group occurred mainly because embryos stopped developing in the first 3 days of culture,

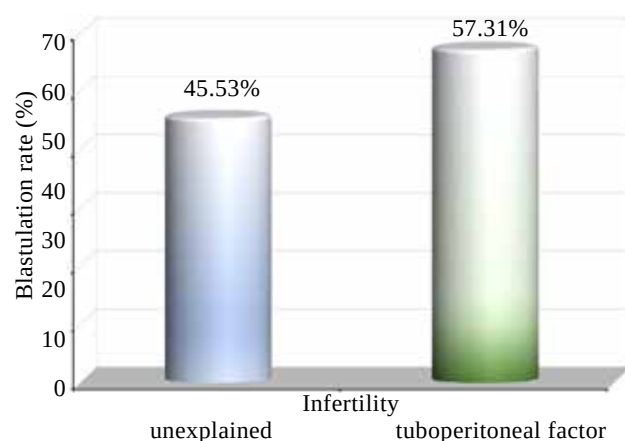


FIGURE 2. Blastulation rate in in vitro fertilization programs in the groups of patients with unexplained infertility and tuboperitoneal factor of infertility. Differences between groups are statistically significant at $p < 0.05$

inclusively. This occurred in unexplained infertility group in 13.8% of cases versus 9% in tuboperitoneal factor of infertility V group. There was no difference between unexplained infertility group (44.7%) and tuboperitoneal factor of infertility group (44.3%) in the percentage of embryos that stopped developing on days 3-5 of culture. This confirms that reduction of blastulation in unexplained infertility was because embryos stopped developing before 3 days of culture, inclusively (Fig. 3).

At lower blastulation rate in unexplained infertility group, there was no difference between unex-

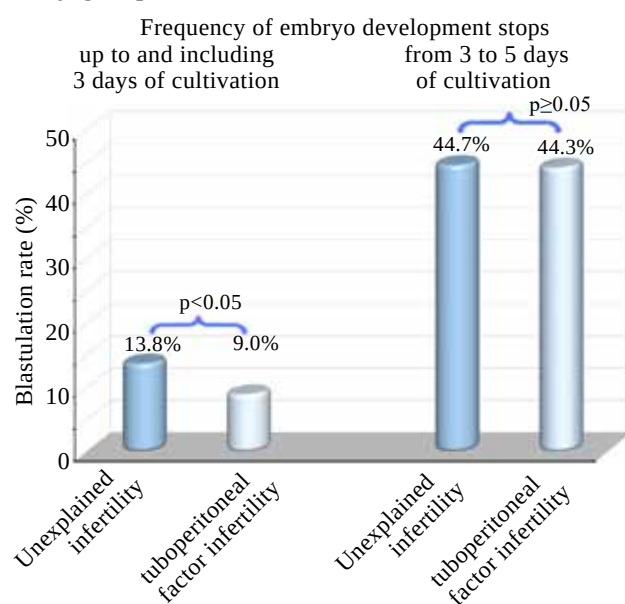


FIGURE 3. Embryo development stops in in vitro fertilization programs in the groups of patients with unexplained infertility and tuboperitoneal factor of infertility. Differences between groups are statistically significant at $p < 0.05$

plained infertility group (55.3%) and tuboperitoneal factor of infertility group (55.7%) in the percentage of blastocyst development from embryos remaining in culture for 3 days. This also confirms that the embryos stop developing before 3 days of culture inclusively in unexplained infertility.

A higher quality of blastocysts was in group unexplained infertility (66.7%) (good-quality blastocysts: >3, AA, AB, BA) versus tuboperitoneal factor of infertility group (45.8%), and there were more in vitro fertilization programs with obtaining good-quality blastocysts versus tuboperitoneal factor of infertility group (65.5 and 43.9%), respectively.

In unexplained infertility, the embryos were transferred before 3 days of culture inclusively less often versus tuboperitoneal factor of infertility [23.75% (on day 1 – 8.75%, on day 3 – 15%) and 30.6% (on day 1 – 5.6%, on day 3 – 25%), respectively]; and the blastocysts were transferred most often versus tuboperitoneal factor of infertility [68.75% (on day 5 – 68.75%) and 58.3% (on day 5 – 58.3%), respectively]. This may in directly reflect the trend for embryo development in unexplained infertility: after 3 days, embryos developed to the blastocyst stage at a normal rate, and these blastocysts were often good-quality blastocysts.

Preimplantation genetic testing for aneuploidy in unexplained infertility (62.7%) was performed in more than half of cases and more often than in cases of tuboperitoneal factor of infertility (14.3%). It was to be expected in cases of unexplained infertility and unsuccessful attempts of getting pregnant naturally and using assisted reproductive technologies. However, according to the results of preimplantation genetic testing for aneuploidy, the frequency of euploid embryos did not differ between unexplained infertility group (41.7%) and tuboperitoneal factor of infertility group (40%), i.e., aneuploidy in embryos is not a conditioning factor for unexplained infertility.

In unexplained infertility group (57.5%), the cancellation rate of embryo transfer in in vitro fertilization programs was higher than in tuboperitoneal factor of infertility group (34.1%) mainly due to fact that the patients underwent preimplantation genetic testing for aneuploidy (47.7%).

Despite the fact, that the rate of cryopreservation of embryos in in vitro fertilization programs was higher in unexplained infertility group (68.1%) versus tuboperitoneal factor of infertility

group (53.7%), there were no differences between the groups in the number of cryopreserved embryos per in vitro fertilization program (unexplained infertility – 3.66 and tuboperitoneal factor of infertility – 3.36) and in the rate of cryopreserved blastocysts (unexplained infertility – 70% and tuboperitoneal factor of infertility – 66.4%).

Integral indicator of blastocysts utilization rate was lower in unexplained infertility group (40%) versus tuboperitoneal factor of infertility group (44%). This reflects decreased blastulation rate in unexplained infertility group, all other conditions being equal [Apter S et al. 2017].

DISCUSSION

The results of the study confirmed the data in a number of publications about delayed healthcare seeking in the in vitro fertilization clinics by patients diagnosed with unexplained infertility, and as a consequence their age was higher [Yücel B et al., 2018; Buckett W, Sierra S, 2019; Siristatidis C et al., 2020]. According to the mathematical model, 2 years after regular sexually active life the frequency of false-positive diagnosis of unexplained infertility increases from 10% in women under 35 years of age to 50% in women over 37 years of age and reaches more than 80% in women over 40 years of age [Apter S et al., 2017; Vogiatzi P et al., 2019]. In our study the age of patients in both groups was limited to 35 years. Nevertheless, the number of women under 35 years of age in tuboperitoneal factor of infertility group was higher than in the other group (96% and 87%, respectively). It is natural that the patients with unexplained infertility underwent medical examination for a long period of time and other methods were used for their treatment. The issue of expectant management and other methods of treatment, primarily intrauterine insemination in cases of unexplained infertility is disputable [Abrahami N et al., 2019]. Intrauterine insemination was performed in 27% of patients with unexplained infertility and had low efficiency: 2% of achieved pregnancies were non-developing pregnancies.

Anamnestic and phenotypic characteristics specific for the patients with unexplained infertility were identified. The conditions that may be categorized as autoimmune diseases: thyroid disorders (autoimmune thyroiditis) and gastrointestinal dis-

orders (chronic gastritis, chronic gastroduodenitis, biliary dyskinesia) were most common in cases of – 31% and 29%, respectively; while the rate of gynecological diseases (endometriosis, adenomyosis, mastopathy, fibroids, chronic endometritis, chronic salpingo-oophoritis, polycystic ovary syndrome and other conditions), pelvic inflammatory disease was significantly high in cases of tuboperitoneal factor of infertility – 66% and 38%, respectively, and the rate of surgical interventions (laparoscopy, hysteroscopy, restoration of tubal patency and other surgical procedures) was 94%. Most commonly these surgical interventions were performed repeatedly, including laparotomy, and were the cause of tuboperitoneal factor of infertility. In unexplained infertility group, uterine fibroids and endometriosis lesions were less common versus tuboperitoneal factor of infertility group (2% and 9%, respectively) and (9% and 13%, respectively), which theoretically can be the cause of infertility. Moreover, excessive body weight and other phenotypic and clinical features, such as polycystic ovaries, hirsutism, insulin resistance, were not specific for women with unexplained infertility.

A number of studies presented the data that patients with unexplained infertility can have low ovarian reserve, which is defined by abnormal levels of anti-Müllerian hormone and antral follicle count in the follicular phase [Yücel B et al., 2018; Bosch E et al., 2021; Kirakosyan E et al., 2021]. This study did not confirm this suggestion. According to the obtained data, ovarian reserve in women with was comparable with ovarian reserve in cases of tuboperitoneal factor of infertility and was normal for the patients of lower age. The only difference was that antral follicle count tended to decrease. This can be explained by the fact that there were no women with polycystic ovaries among the patients with unexplained infertility versus the patients with tuboperitoneal factor of infertility.

Therefore, the clinical and anamnestic characteristics and parameters of the reproductive system were quite normal in patients with unexplained infertility, for this reason, at this stage of the study, there was no answer to the question, why pregnancy did not occur.

In this respect, the embryological stage of in vitro fertilization programs was investigated. The obtained data indicated that in cases of unexplained infertility, the number of mature oocytes

was sufficient, the fertilization rate was normal. The results of our study showed that statistically significant difference of the embryological stage of in vitro fertilization programs was a low blastulation rate in patients with unexplained infertility versus patients with tuboperitoneal factor of infertility (45.53% and 57.31%, respectively) due to a low quality of oocytes and/or genetic or epigenetic factors that have an impact on the process of early embryogenesis [Larbuissou A et al., 2017; Bosselut H et al., 2021]. Investigation of possible causes and factors of impaired early embryogenesis is a relevant objective of modern science [Mansour R et al., 2017; Sfakianoudis K et al., 2021; Kirakosyan E et al., 2022a].

The low blastulation rate could be an indirect sign of impaired early embryogenesis and, therefore, an indication for early use of in vitro fertilization with purpose of achieving pregnancy. Limited information is presented in literature about embryological parameters in patients with unexplained infertility. The existing data suggesting that the rate of fertilization failures after in vitro fertilization in patients with unexplained infertility reaches 43% and exceeds the rate of fertilization failures after in vitro fertilization/intracytoplasmic sperm injection is controversial and were not confirmed by our study [Bosselut H et al., 2021]. In this study, fertilization was achieved in unexplained infertility group in 67% of cases using intracytoplasmic sperm injection, and this largely reflects generally accepted clinical practice. However, the comparison between in vitro fertilization and in vitro fertilization/intracytoplasmic sperm injection showed that the rates of fertilization were similar.

Decreased blastulation rate and high rate of embryos that stopped developing before 3 days of culture in in vitro fertilization programs indicate impaired early embryo development and reflect regularity of this process in unexplained infertility. This can be regarded as a possible cause of infertility in unexplained infertility, and, therefore, additional tests that are often recommended for married couples and long-term empiric treatment cannot be justified. As has been previously shown, statistically, the average duration of infertility was significantly higher in unexplained infertility group – 5.65 years than in tuboperitoneal factor of infertility group – 4.75 years [Kirakosyan E et al., 2022b].

It is believed that embryonic genome activation occurs by the four-cell stage. For this reason, it is probable that embryo stops developing at cleavage stage (on days 1-3) due to oocyte factor, and impaired embryo development between morula compaction and blastocyst formation (on days 4-6) occurs due to embryo genome [Sfakianoudis K et al., 2021].

Normal fertilization rate, decreased blastulation rate due to higher incidence of stopping of embryonic development up to 3 days of culture and, at the same time, high quality of obtained blastocysts explain the pathogenesis of unexplained infertility. Conception in these married couples occurs with normal frequency, the embryos stop developing most often up to 3 days of culture, blastocysts are produced less often, but they are good-quality blastocysts. For this reason, pregnancy may occur, but the time for getting pregnant may be prolonged. At the same time, the age and increasing age-related comorbidity of patients with unexplained infertility lead to an additional restriction of realization of their reproductive function. *in vitro* fertilization reduces the time to obtain good-quality blastocysts and, accordingly, to become pregnant, i.e., this is a pathogenetic approach to unexplained infertility treatment technique. All this proves that it is reasonable that the patients with unexplained infertility should undergo *in vitro* fertilization as early as possible with good-quality embryo transfer (>3, AA, AB, BA) on day 5-6 of culture.

No differences between unexplained infertility and tuboperitoneal factor of infertility groups in the rates of euploid embryos detected by preimplantation genetic testing for aneuploidy indicate that routine preimplantation genetic testing for aneuploidy in cases of unexplained infertility is inexpedient. This increases the time before embryo transfer and, accordingly, for getting pregnant. Moreover, trophoctoderm biopsy procedure may reduce the implantation rate of *in vitro* fertilization due to a possible effect of invasive intervention on proper development and function of the placenta [Cimadomo D et al., 2016; Zacchini F et al., 2017].

Oocyte is a major factor determining human embryonic development [Larbuissou A et al., 2017]. The quality of oocyte is the ability of oocyte to be successfully fertilized and ensures normal embryo development at early stages. The existing evidence does not give grounds for the conclusion that the

cause of unexplained infertility is poor-quality oocytes with a satisfactory number of mature oocytes – more than 6 per puncture (on average 10.8 in our study), satisfactory fertilization rate (76.5 % in our study), possibility of cryopreservation of embryos in 30% of *in vitro* fertilization attempts (68.1% in our study) and the frequency of early pregnancy losses comparable to the general population (9.2% in medical history of patients in our study) [Boselut H et al., 2021; Bachurin A et al., 2022]. At the same time, poor oocyte quality as the cause of unexplained infertility cannot be ruled out [Kirakosyan E et al., 2022a].

CONCLUSION

This study identified the clinical characteristics and embryological parameters in *in vitro* fertilization programs for women with unexplained infertility. A “clinical portrait” of women with unexplained infertility was described and specific features of infertility were identified versus the women with tuboperitoneal factor of infertility. Low blastulation rate was considered to be the endpoint in *in vitro* fertilization treatment programs, and it was significantly low in the group of women with unexplained infertility.

In-depth analysis of the embryonic stage of *in vitro* fertilization programs showed, that low blastulation rate in unexplained infertility was mainly due to stopping of embryonic development up to 3 days of culture. At the same time, the morphological assessment showed that the quality of blastocysts was higher in unexplained infertility group compared to tuboperitoneal factor of infertility group; and preimplantation genetic testing for aneuploidy showed a similar frequency of detection of euploid embryos.

The obtained results suggest that it is inexpedient to recommend the married couple with unexplained infertility to undergo expanded diagnostic testing and empirical treatment, and early *in vitro* fertilization with good-quality embryo transfer (>3, AA, AB, BA) on day 5-6 of culture is necessary.

Further research is needed to investigate the molecular mechanisms of impairment in early stages of embryogenesis, primarily including the study of gamete quality in patients with unexplained infertility [Kirakosyan E et al., 2022c].

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