OBSTETRICS AND GYNAECOLOGY

ASSISTED HATCHING IN CRYOPRESERVATION PROTOCOLS IN PATIENTS WITH TUBOPERITONEAL INFERTILITY

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ABSTRACT

At present, the problem of increasing the effectiveness of programs of assisted reproductive technologies and successful infertility treatment is still relevant. Assisted hatching used in the devitrificated embryo transfer facilitates the exit of the embryo from the pellucide zone. Yet the clinical efficacy of assisted hatching is relevant and debatable. There are no clear indications for the use of this technology, and no groups of patients have been identified.

The aim of the study. *To assess the effectiveness of laser hatching in the frozen-thawed embryo transfer programs in patients with tuboperitoneal infertility.*

Materials and methods. *We examined 300 women with tuboperitoneal infertility who had their embryos frozen for transfer. Inclusion criteria: age from 18 to 35 years; tuboperitoneal infertility; embryos cryopreserved for transfer. Exclusion criteria: age more than 36 years; other infertility factors. Women were divided into 2 groups: group 1 – women who had a frozen-thawed embryo transfer with preliminary laser hatching (n = 137); group 2 – control group (n = 163).*

Results. *There were no differences between the groups in the mean age, body mass index and the age at menarche. According to the results of the embryological stage, there were also no differences in the number and quality of frozen embryos. The pregnancy rate in the group with preliminary laser hatching was 44.5 %, which is significantly higher than in the control group (42.3 %; $p \leq 0.001$). We also found statistically significant differences in pregnancy outcomes: in the frequency of spontaneous miscarriages – 13.1 % and 20.2 % respectively ($p \leq 0.001$), in the frequency of term deliveries – 30.7 % and 22.1 % respectively ($p \leq 0.001$).*

Conclusion. *In our study, the using laser hatching in women with tuboperitoneal infertility positively affected the embryos implantation in the cryopreservation protocols. Pregnancy and live birth rates are higher after using hatching technology, and the frequency of miscarriages up to 12 weeks is lower. This provide an opportunity to further study the effect of hatching on long-term outcomes, such as gestation course and childbirth.*

Key words: *assisted reproductive technologies, cryopreservation protocol, frozen-thawed embryo, cryopreservation, assisted hatching*

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ПРИМЕНЕНИЕ ВСПОМОГАТЕЛЬНОГО ХЭТЧИНГА В КРИОПРОТОКОЛАХ У ПАЦИЕНТОК С ТРУБНО-ПЕРИТОНЕАЛЬНЫМ БЕСПЛОДИЕМ

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РЕЗЮМЕ

В настоящее время проблема эффективного преодоления бесплодия в программах экстракорпорального оплодотворения остаётся по-прежнему актуальной. Технология вспомогательного хэтчинга, используемая при переносе девитрифицированного эмбриона, направлена на облегчение высвобождения эмбриона из блестящей оболочки. Однако вопрос его клинической эффективности остаётся крайне актуальным и противоречивым.

Цель исследования. *Оценка эффективности применения лазерного хэтчинга в программах с переносом размороженного эмбриона у женщин с трубно-перитонеальным бесплодием.*

Материалы и методы. *Было обследовано 300 женщин, страдающих трубно-перитонеальным бесплодием, которые имели криоконсервированные эмбрионы. Критерии включения: возраст от 18 до 35 лет включительно; трубно-перитонеальное бесплодие; наличие криоконсервированных эмбрионов для переноса. Критерии исключения: возраст 36 лет и старше; наличие других факторов бесплодия. Далее были сформированы две группы: группа 1 – женщины, у которых перенос размороженных эмбрионов выполнялся с проведением предварительного лазерного хэтчинга (n = 137); группа 2 – группа контроля (n = 163).*

Результаты. *Группы сравнения не различались по среднему возрасту, индексу массы тела, возрасту менархе. По результатам эмбриологического этапа также не выявлены различия по количеству и качеству замороженных эмбрионов. Частота наступления беременности в группе исследования с проведением лазерного хэтчинга составила 44,5 %, что статистически значимо выше, чем в группе контроля (42,3 %; p ≤ 0,001). Также нами были выявлены статистически значимые различия в исходах беременностей: в частоте самопроизвольных выкидышей – 13,1 % и 20,2 % соответственно (p ≤ 0,001), срочных родов – 30,7 % и 22,1 % соответственно (p ≤ 0,001).*

Заключение. *В нашем исследовании применение лазерного хэтчинга благоприятно повлияло на имплантацию в криопротоколах. Однако связь между хэтчингом и долгосрочными исходами, такими как течение беременности и роды, требует дальнейшего изучения.*

Ключевые слова: *вспомогательные репродуктивные технологии, криопротокол, размороженный эмбрион, криоконсервация, вспомогательный хэтчинг*

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RELEVANCE

At present, the problem of increasing the efficiency of programs of assisted reproductive technologies and successful infertility treatment is still relevant. Data published in the 26th annual report of the Register of Assisted Reproductive Technologies (ART) of the Russian Association of Human Reproduction (RAHR) show that the pregnancy rate in in vitro fertilisation (IVF) programs in 2020 was: per cycle – 28.9 % (in 2019 –32.3 %), per puncture – 30.0 % (in 2019 – 33.3 %), per embryo transfer – 34.8 % (in 2019 – 38.5 %) [1]. In programs with frozen-thawed embryo transfer, the pregnancy rate per cycle was 41.3 % (41.8 % in 2019) and per embryo transfer was 42.1 % (43.0 % in 2019) [1]. Despite significant progress in the field of assisted reproductive technologies, it is impossible to improve the outcome of ART programs without studying the mechanisms of implantation.

The outcome of ART programs is influenced by many factors. The main ones are endometrial condition, embryo quality and delivery to the implantation site [2, 3]. However, during the preimplantation development stage, the embryo is inside a glycoprotein shell, which is called the zona pellucida (pellucid zone) [4, 5]. Failure of the embryo to emerge from the zona pellucida often results in failure in ART programs and spontaneous pregnancy does not occur.

The human oocyte is surrounded by the zona pellucida shell composed of specific glycoproteins. After fertilization, blocking mechanisms are triggered in the zona pellucida and plasma membrane of the oocyte to prevent the penetration and fusion of additional spermatozoa [6, 7]. After fertilization, the zona pellucida shell persists and surrounds the developing human embryo for a few more days. Then, the embryo emerges from the shell and establishes cell contacts between the trophectoderm and the endometrial epithelium for subsequent implantation [8]. In embryology, the term «hatching» refers to the emergence of an embryo from the zona pellucida shell: spontaneous hatching occurs at the blastocyst stage, when the shell ruptures and the blastocysts emerge through the resulting gap. The zona pellucida rupture is caused by the following factors: release of proteolytic enzyme – cathepsin – by the cells of trophectoderm, which in turn dissolves the area of the shell; mechanical rupture of the zona pellucida by a blastocyst due to an increase in size [9, 10].

At the embryological stage, various factors may affect a blastocyst, such as the composition of the culture medium, intracellular zinc concentration in the oocyte, impaired synthesis of metalloproteinases and the use of cryopreservatives for the embryo freezing, leading to thickening of the zona pellucida, which necessitates preliminary assisted hatching [11–13].

Assisted hatching is an embryological technique that aims to increase the embryo implantation rate by eliminating the cause of incomplete hatching. Several types of assisted hatching are used in embryological practice: mechanical, laser-assisted, chemical or enzymatic. During mechanical hatching, the zona pellucida shell is pierced with a micro-needle, but there is a risk of rupture or loss of blastomeres and there may be an increased incidence of multiple pregnancies [12–14]. During chemical hatching, an acid is applied to the shell, which if the technique of the procedure is not followed can be destructive to the blastomeres adjacent to the hole made. The zona pellucida shell dissolves on contact with acid, so the embryo is immediately removed and washed several times to remove any traces of acid [14].

The laser is ideal for microsurgical procedures because the energy is easily focused on the target area, creating a controlled and precise orifice that is coordinated between operators. Using an optical lens, the laser beam is directed tangentially to the embryo through the zona pellucida in non-contact mode or touches the embryo and performs hatching in contact mode [14]. Femtosecond laser pulse technology is currently used in cell surgery. According to the study of M.M. Rakityansky et al., the use of femtosecond laser pulses allows for precision perforation of the zona pellucida without affecting its cells. A femtosecond laser scalpel-tweezers are used to perform laser-assisted hatching. An optical mammalian embryo biopsy technique has been developed. It allows non-contact isolation of material from the embryo for preimplantation diagnosis of its condition. The results of the study show that about 90 % of embryos subjected to such operations retained the ability to develop to the blastula stage [15].

A. Alteri et al. who compared mechanical, chemical and laser-assisted hatching reported the superiority of the latter [16]. In the study by C. Liu et al. use of laser-assisted hatching during transfer of a single frozen-thawed blastocyst shows higher rates of implantation, pregnancy and live birth [17].

Despite the large number of existing embryological techniques, the issue of clinical efficiency of programs of cryopreserved embryo transfer remains highly relevant and controversial. Considering the above arguments, the aim of the study was to evaluate the efficiency of laser hatching in programs with frozen-thawed embryo transfer among female patients with tuboperitoneal infertility.

MATERIALS AND METHODS

The study was conducted at the ART department of the Regional Perinatal Center of the State Budgetary Healthcare Institution «Irkutsk Regional Clinical Hospital, winner of the "Mark of the Honor" award from 2018 to 2021. A total of 300 women receiving ART treatment for tuboperitoneal infertility (ICD-10: N97.1) were examined. After the IVF program, the patients' embryos were vitrified for further transfer. The inclusion criteria were: age range from 18 to 35 years inclusive; tuboperitoneal infertility; embryos vitrified for transfer. Exclusion criteria: age 36 years and older; other infertility factors; endometriosis; uterine anomalies; absence of vitrified embryos; male

infertility; use of donor material (donor oocytes, sperm and embryos).

Patients with cryopreserved embryos were divided into two groups depending on the use of laser-assisted hatching: group 1 – women who had a frozen-thawed embryo transfer with preliminary laser hatching (n = 137); group 2 – control group which consisted of women who had a frozen-thawed embryo transfer without hatching (n = 163). All patients gave a voluntary informed consent to participate in the study. The work with the patients was carried out in accordance with the ethical standards of the World Medical Association Declaration of Helsinki (ed. 1964, 2012). The conduct of this study was approved by the local ethical committee of the Irkutsk State Medical Academy of Postgraduate Education – Branch Campus of the Russian Medical Academy of Continuing Professional Education of the Ministry of Health of Russia (Minutes No. 12 dated 14.11.2017).

Clinical and medical history data, IVF program and embryological stage, cryopreservation protocol and pregnancy rate of the compared groups were studied after the performed manipulations.

Controlled ovarian stimulation in IVF cycles was performed according to a protocol with gonadotropin-releasing hormone antagonists. The average doses of gonadotropins used were not statistically different between groups. The ovulation trigger is chorionic gonadotropin at a dosage of 6500 IU. The method of fertilization is IVF or intracytoplasmic sperm injection (ICSI).

At the cleavage stage, embryo quality was assessed according to the classification of J. Lens et al., at the blastocyst stage – according to the classification of D. Gardner et al. (1999). Embryos were vitrified (ultra-fast cryopreservation) using Kitazato reagent kit (Japan). Thawing was carried out according to the manufacturers' recommendations. Preparation of the endometrium for thawed embryo transfer was performed with step-by-step administration of estrogen and progesterone preparations according to the days of embryo culturing. In all cases, transfer of one or two embryos of excellent and good quality was performed when endometrial thickness of 8 mm or more was achieved. The efficiency of this method was assessed by pregnancy rate and long-term outcomes (spontaneous abortion and term birth).

Statistical processing was carried out with preliminary assessment for compliance with the normal distribution (the Gaussian distribution), then by nonparametric methods using the Statistica v. 10.0 basic package (StatSoft Inc., USA). The parametric Student's test (t-test) was used to test the statistical hypothesis of equality of two independent samples in the case of normally distributed continuous variables. The nonparametric Mann – Whitney test was used for pairwise comparison of groups in the case of distribution of values different from the normal distribution. The χ^2 test, the χ^2 test with Yates's correction for continuity and the two-tailed Fisher exact test were used in the analysis of 2 × 2 contingency tables. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

At the first stage, we analyzed the clinical and medical history data. The comparison groups did not differ in the patients' average age, body mass index and age at menarche. The average duration of infertility in the studied patients also had no statistical differences and was 5.1–5.9 years ($p = 0.6$). A history of pelvic inflammatory diseases was found in more than 95 % of cases (group 1 – 95 % of cases; group 2 – 96.6 % of cases; $p = 0.9$); reconstructive surgeries on fallopian tubes were performed in more than 70 % of cases (group 1 – 77 % of cases; group 2 – 77.5 % of cases; $p = 0.5$), but no statistically significant differences were found.

In the study groups, secondary infertility was more frequent than primary infertility: in group 1, primary infertility was registered in 47.7 % and secondary infertility in 52.3 % of cases; in group 2, in 38 % and 62 % of cases, respectively. One third of all women studied had a history of childbirth (31.1 % and 32.7 %, respectively). Half of the women had medical abortions (50.9 % and 51.7 % respectively). It is important to note that half of the subjects had a history of ectopic pregnancies (55.2 % and 53.2 %, respectively; $p = 0.4$), which led to fallopian tube surgery, but the differences were not statistically significant.

Hormonal panel analysis (Table 1) showed that hormone values were within reference values, but the control group had statistically higher levels of follicle-stimulating hormone (FSH) and progesterone than the assisted hatching group (7.2 ± 1.9 and 6.3 ± 1.9 mIU/ml, respectively ($p = 0.012$); 31.2 ± 22.1 and 24.5 ± 16.3 nmol/L, respectively ($p = 0.012$)). The control group also differed statistically significantly in low Anti-Müllerian hormone (AMH) levels (3.2 ± 1.5 ng/ml) from the assisted hatching group (5.4 ± 2.6 ng/ml; $p < 0.001$) and had a statistically significantly lower number of antral follicles (6.1 ± 2.4 and 8.6 ± 3.9, respectively; $p < 0.001$), but all women studied had adequate ovarian reserve and number of embryos for cryopreservation.

Embryological stage and pregnancy outcomes were further analyzed in our study and the data are presented below (Table 2). Spontaneous abortions were defined as pregnancies that ended before 12 weeks. Term birth was defined as birth from 37 weeks onwards.

According to the results of the embryological stage (Table 2), the comparison groups did not differ in the number of cryopreserved and thawed embryos and embryo quality. Optimal endometrial thickness at the time of embryo transfer was also achieved in the study groups, but the differences were not statistically significant.

The pregnancy rate and outcomes deserve special attention: pregnancy occurred in 44.5 % of cases in the study group with laser-assisted hatching, in the control group – in 42.3 % of cases ($p < 0.001$); spontaneous abortion rates were 13.1 % and 20.2 %, respectively ($p < 0.001$). In group 1, 30.7 % of pregnancies ended in birth, in the control group – 22.1 %; the differences were statistically significant ($p < 0.001$). Therefore, preliminary assisted hatching increased the pregnancy rate in the study group and favorably influenced the pregnancy outcome compared to the control group.

TABLE 1

CLINICAL AND ANAMNESTIC CHARACTERISTICS IN THE STUDIED GROUPS

Indicators	Group 1 (*n* = 137)	Group 2 (*n* = 163)	*p value*
	M ± SD; Me (25th; 75th percentiles)		
Baseline FSH, mIU/ml	6.3 ± 1.9; 6.3 (0.6; 9.1)	7.2 ± 1.9; 7 (2.7; 9.4)	0.012*
Baseline LH, mIU/ml	6.7 ± 4.3; 6.1 (0.9; 15.6)	5.9 ± 2.8; 5.4 (1.3; 11.5)	0.4
Baseline progesterone, nMol/L	24.5 ± 16.3; 16.1 (0.1; 160)	31.2 ± 22.1; 30.6 (0.2; 84)	0.012*
Baseline AMH, ng/ml	5.4 ± 2.6; 4.9 (1.2; 12.9)	3.2 ± 1.5; 3.3 (0.8; 9.6)	< 0.0001*
Left ovary volume, cm^3	8.3 ± 7.3; 7.1 (1.9; 13.4)	7.4 ± 4.5; 6.7 (1.9; 16.6)	0.1
Right ovary volume, cm^3	11.1 ± 7.1; 8.3 (0.5; 14)	8.1 ± 4.1; 7.6 (0.7; 13.8)	0.1
Number of antral follicles	8.6 ± 3.9 9.0 (5.5; 12.0)	6.1 ± 2.4 5.5 (4.5; 7.0)	<0.001

Note. LH – luteinizing hormone; * – $p \leq 0.05$.

TABLE 2

CRYOPRESERVATION PROTOCOL PARAMETERS IN THE STUDIED GROUPS

Indicators	Group 1 (n = 137)	Group 2 (n = 163)	p value
Vitrification on the 3th day of cultivation, *n* (%)	20 (14.6 %)	21 (12.9 %)	0.7
Vitrification on the 4th day of cultivation, *n* (%)	49 (35.8 %)	74 (45.4 %)	0.2
Vitrification on the 5th day of cultivation, *n* (%)	68 (49.6 %)	68 (41.7 %)	0.4
Number of thawed embryos, M ± SD; Me (25th; 75th percentiles)	1.8 ± 0.4; 2 (1; 2)	1.8 ± 0.3; 2 (1; 2)	0.4
Thawing percentage, M ± SD; Me (25th; 75th percentiles)	85.4 ± 20.9; 100 (33.3; 100)	80.3 ± 23.1; 83.4 (33.3; 100)	0.5
«Day-to-day» cryotransfer, *n* (%)	67 (48.9 %)	61 (37.4 %)	0.2
Completing of cryopreserved embryo growing, *n* (%)	70 (51.1 %)	102 (62.6 %)	0.2
Cryotransfer of 4-day old embryos (morula), *n* (%)	8 (5.8 %)	8 (4.9 %)	0.7
Cryotransfer of 5-day old embryos (blastocyst), *n* (%)	129 (94.2 %)	155 (95.1 %)	0.9
Good quality embryos (for transfer), *n* (%)	60 (43.8 %)	56 (34.4 %)	0.2
Fair quality embryos, *n* (%)	75 (54.7 %)	103 (63.2 %)	0.4
Poor quality embryos, *n* (%)	2 (1.5 %)	4 (2.5 %)	0.5
Endometrial thickness at the time of transfer, M ± SD; Me (25th; 75th percentiles)	10.2 ± 1.4; 10 (8; 12)	10.2 ± 1.1; 10 (8.3; 12)	0.3
Pregnancy rate, *n* (%)	61 (44.5 %)	69 (42.3 %)	< 0.0001*
Spontaneous abortions, *n* (%)	18 (13.1 %)	33 (20.2 %)	< 0.0001*
Term births, *n* (%)	42 (30.7 %)	36 (22.1 %)	< 0.0001*

Note. * – $p \leq 0.05$.

CONCLUSION

Despite the successful development of methods of assisted reproductive technologies (ART), the issues of improving the efficiency of infertility treatment remain relevant. The search for new solutions leads to the introduction of new techniques into routine embryological practice. Currently, laser-assisted hatching is widely used in cryopreservation protocols, but the need and indications for it are not fully studied.

In our study, preliminary laser-assisted hatching increased implantation in cryopreservation protocols and favorably influenced the course of pregnancy and birth among patients with tuboperitoneal infertility. However, the correlation between hatching and long-term outcomes such as pregnancy course and birth requires further study.

Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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