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Methods of preparation of endometrium in patients with ovarian hyperstimulation syndrome before the transfer of vitrified / warmed embryos

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Abstract

The aim of the study was to develop a personified method of endometrium preparation for the transfer of vitrified / warmed embryos in women with the development of OHSS in IVF programs. 39 women with early OHSS after IVF, who were undergoing personalized correction of the morphofunctional state of the endometrium, and 39 patients with baseline OHSS II group undergoing standard therapy were examined and treated. Control group consisted of women without OHSS in the IVF cycle. Personalized correction of the morphofunctional state of the endometrium includes traditional hormonal replacement therapy and addition of adjuvants: preparation with extract of horse chestnut and thiamine hydrochloride, vitamin E, serratiopeptidase, myo-inositol, the product of proteolysis of the placenta of cattle, L-arginine aspartate. The application of the developed method for the preparation of endometrium for the transfer of vitrified / warmed embryos by the developed method contributed to the prolongation of pregnancy and the increase in the number of live births in 2,11 times (OR 3.17; 95% CI 1.20-8.39; p<0,02). The increase in absolute benefit (therapeutic benefit) of the proposed person-adjusted correction is 25.64% (95% CI 5.12-46.16%), an increase in relative benefit corresponds to a clinically significant effect of 111.10% (95% CI 9.40 – 307.40 %), the number of patients to be treated is 3.90 (95% CI

2.17-19.52). The developed method is effective for the preparation of endometrium for the transfer of vitrified / warmed embryos.

Key words: in vitro fertilization, ovarian hyperstimulation syndrome, cycle segmentation, vitrified / warmed embryos, method of endometrium preparation, live births.

In case of high risk or development of ovarian hyperstimulation syndrome (OHSS), the *in vitro* fertilization (IVF) treatment cycle can be segmented, all embryos are cryopreserved without the transfer of a fresh embryo. After restoring the morphofunctional state of the ovaries, the embryos are thawed and transferred to the uterus in the natural cycle, a cycle of replacement hormone therapy or stimulated cycle.

A meta-analysis from 11 observational studies has shown that pregnancy after the transfer of vitrified / warmed embryos is associated with a lower risk of preterm delivery, low birth weight and perinatal mortality than pregnancy from transferred a fresh embryo [16, 17]. Subsequently, other studies comparing the two procedures showed better pregnancy rates through the transfer of vitrified / warmed embryos [19, 22].

The causal relationships between the periimplanting environment and the perinatal outcomes are confirmed by several studies demonstrating that the adverse effects of the periimplantation medium are most significant in those patients who are most reactive to COS [9, 13, 14]. A recent pilot study by AN Imudia.et al. (2013) has shown that in patients at high risk of OHSS who have chosen cryopreservation of all embryos, preeclampsia and birth weight of children, defined as <10% for gestational age, were lower than those who decided to continue embryo transfer [13]. This group also showed higher rates of preeclampsia and the birth of children of low gestational age in patients with a level of E_2 greater than 90 percentile, indicating that an energetic response to superovulation may be associated with a higher risk of adverse outcomes of pregnancy (OR for the birth of children with low weight - 9.40, 95% DI 3.22-27.46; OR for preeclampsia - 4.79, 95% DI 1.55-14.84) [12].

The aim of the study was to develop a personified method of endometrium preparation for the transfer of vitrified / warmed embryos in women with the development of OHSS in IVF programs.

Materials and methods

A randomized controlled trial of the effectiveness of the developed measures of personified correction of the morphofunctional state of endometrium compared with the standard approach was conducted. 39 women of the I group (experimental) with early OHSS

after IVF, who were undergoing personalized correction of the morphofunctional state of the endometrium, and 39 patients with baseline OHSS II group undergoing standard therapy were examined and treated. Control group K consisted of 30 women without OHSS in the IVF cycle. All groups were homogeneous according to age, anthropometric data, gynecological, reproductive, infectioous and somatic anamnesis, duration of infertility, number of IVF attempts, uterine and ovarian size, ovarian reserve and hormonal status.

The criteria for inclusion of patients in the OHSS group were: the presence of infertility and the development of early moderate or severe OHSS in the IVF cycle during COS with antagonists of gonadotropin-releasing-hormone (antGnRH); informed consent of women to participate in the study. Exclusion criteria are the presence of ovarian tumors, diabetes mellitus, acute infectious diseases.

We used the following COS methodology. From the 2nd day of the menstrual cycle, gonadotropins were started in the average starting dose of FSH 75-150 IU. At 6-8th days, they performed a sonography, when the follicle was detected > 14 mm, antGnRH was administered at a dose of 0.25 mg / day for 4-5 days and gonadotrophin stimulation continued until the leading follicle reached > 18 mm in diameter and at least three follicles sizes > 16 mm. Then a trigger of ovulation was prescribed – agonist GnRH (aGnRH) in a dose of 0.1-0.2 mg; after 34-36 hours, the egg collection and their fertilization were performed. The resulting embryos were vitrified. Women were withdrawn from the fertilization treatment cycle and received the necessary treatment for OHSS. From the first menstruation after the treatment cycle, the endometrium was preparated.

In the comparison group II, endometrial preparation was carried out according to the traditional method as follows: three months after the IVF cycle, replaced cyclic hormone therapy was performed; on the 21st day of the third menstrual cycle, 3.75 mg of tryptoreline was injected subcutaneously, after 14 days, the use of transdermal estrogens was started for 12-14 days. After reaching the thickness of the endometrium of 10-12 mm, they began taking progesterone preparations. On P+7-P+8 day, two frozen embryos were transferred at the stage of the blastocyst. Before transplantation, if necessary, pre-implantation diagnosis was performed to evaluate the genetic material and the numerical chromosome set of the embryonic cells.

For transfer, use soft, biologically inert (non-toxic) catheters, unable to damage the embryo, which made it possible to quickly and delicately place the embryo in the uterine cavity. Penetration into the uterus for the purpose of subcutaneous injection was carried out in two stages. The first – a cavernous catheter, more rigid, passed the cervical canal. The second

– a soft catheter, through the opening in the first catheter, was injected with the germ along with the fluid for cultivation in the uterus cavity. We tried to carry the transfer very smoothly, without sharp movements. We prescribed vaginal forms of progesterone to support implantation and post-implantation during pregnancy.

In group I, the preparation of endometrium was carried out according to the developed methodology, which was conducted similarly to that in group II, but with the addition of adjuvants: 12-15 drops of the preparation, 100 g of which contains 5.4 g dry extract of horse chestnut (1,0 g of triterpenglikozid (triterpensaponin), referenced to escine), 0.5 g of thiamine hydrochloride; drops were prescribed 3 times a day, before meals, with a small amount of fluid, a course of three months; vitamin E in capsules of 400 mg once a day after eating with enough fluid in the second half of the menstrual cycle, three cycles; serratiopeptidase in 10 mg pills 2 times a day, washed with a glass of water for a month; preparations of myo-inositol 2000 mg twice a day for three months; the course of intramuscular injection of the drug, 1 ml of solution containing the product of proteolysis of the placenta of cattle - 15 mg, for a course of 10 injections in a day; oral solution, 5 ml containing L-arginine aspartate 1 g (L-arginine - 0.57 g, acid aspartic acid - 0.43 g) per 5 ml per os 3-6 times a day, course of treatment 15 days.

The processing and analysis of statistical information was carried out using the IBM SPSS Statistics 22 (Statistical Package for the Social Science) software.

Results and discussion

The evaluation of reproductive results in patients with OHSS was performed depending on the method of endometrial preparation before the transfer of vitrified / warmed embryos (Table).

Table

Clinical results of IVF in patients with OHSS after the transfer of vitrified-warmed embryos, depending on the methodology of endometrium preparation

Group	Clinical Pregnancy		Live birth		Miscarriage		Ectopic pregnancy	
	n	%	n	%	n	%	n	%
I (n=39)	23	58,97	19	48,72 ^{II}	4	10,26	0	0,00
II (n=39)	18	46,15 ^{k}	9	23,08 ^{k,I}	8	20,51	1	2,56
K (n=30)	30	100	24	80,00	5	16,67	1	3,33

Notes. $^{k, I, II}$ - statistically significant difference with the indicators of groups K, I, II, $p < 0,05$.

At the incidence of pregnancy, the groups I studied (58.97% (23) and II (46.15% (18)) did not differ statistically significantly. The number of cases of live birth in group I (48.72% (19)) exceeded that in the group II (23,08%) in 2,11 times (OR 3,17±0,50; 95% CI 1,20-8,39; p<0,02). Increasing the absolute benefit (therapeutic benefit) of the proposed personified correction was 25.64% (95% CI 5.12-46.16 %), the increase in relative benefit corresponded to a clinically significant effect – 111.10% (95% CI 9.40-307.40 %), the number of patients, which should be treated, – 3.90 (95% CI 2.17-19.52).

The number of miscarriages after IVF in patients with OHSS after the transfer of vitrified / warmed embryos with the preparation of the endometrium according to the developed method was the smallest and was 10.26% (4), whereas in the group II – 20.51% (8) and in the group K 16,67% (5). Ectopic pregnancies were observed in 2.56% (1) of Group II patients and in 3.33% (1) of patients in the K group; in group I, no such cases were reported.

The results obtained can be explained by the therapeutic effect of the developed method of preparing a patient with a transmitted OHSS to the transfer of vitrified / warmed embryos.

The preparation containing the dry extract of horse chestnut Aesculus hippocastanum, the active substance of which is escine, has a pronounced venotonic, anti-inflammatory and anti-edema activity in diseases of the veins. Clinical reports on the vascular efficacy of escine pay particular attention to improving microcirculation, reducing vascular permeability, increasing venous tone and venous return, all that reduces edema [7]. It was suggested that the observed effects are the result of the protection of endothelial cells from hypoxia and inflammatory stimuli. In fact, as shown in pre-clinical studies, β-escine retains ATP in the absence of oxygen, reduces the response of histamine, and releases cytokines, suppresses serotonin-induced capillary permeability [8], suppresses extravasation and migration of leukocytes [6] and preserves the morphology of endothelial cells [15]. It is also worth noting the data indicating the antioxidant potential of β-escine [10, 18] and its inhibitory effect on hyaluronidase, the enzyme is involved in the pathogenesis of chronic venous insufficiency [20]. In later inflammatory work, the softening properties β-escine was associated with its modulating effect on the tumor necrosis factor-α-mediated inflammatory route [21].

Escine is able to break the formed positive feedback, restoring normal hemocytes in venous blood vessels and preventing the development of decompensation of diseases. Anticoagulant and anti-aggregant action of the drug make its use effective for the prevention of thrombosis. The presence of an anti-inflammatory, anti-edema, capillary-resistant effect,

and the ability to reduce the permeability of the plasmolymphatic barrier provides rapid elimination of symptoms [3], which is especially important in the development of OHSS.

Vitamin E and L-arginine improve the function of the luteal body and increase the endometrium in patients by increasing the flow in the uterine artery [5].

Serratiopeptidase is a natural proteolytic enzyme isolated from the non-pathogenic Serratia E15 bacterial bacteria. It has fibrinolytic, anti-inflammatory and anti-edema activity [11]. Serratiopeptidase binds in a ratio of 1:1 to α2-macroglobulin, which masks its antigenicity but retains its enzymatic activity. Serratiopeptidase slowly passes into the exudate in the inflammation center and gradually it decreases in blood. With the help of hydrolysis, bradykinin, histamine and serotonin seratoypeptidase directly reduces dilatation of capillaries and control their permeability. Serratiopeptidase blocks the plasma inhibitors, thereby contributing to its fibrinolytic activity.

Mio-inositol is widespread in human tissues and cells and is a precursor of phosphoinositides involved in signal transduction by stimulating membrane receptors and other secondary messengers, including diacylglycerol and inositol triphosphate [23]. The importance of myo-inositol and its derivatives to maintain the physiological flow of a wide range of processes in the human body was established as a result of systematic analysis of more than 37 thousand publications. The main function of myo-inositol and its derivatives is to contribute to the intracellular signaling and to ensure the functioning of such important receptors as insulin receptors, reproductive hormones, growth factors, catecholamines, and others. The derivatives of myo-inositol interact with specific proteins that are involved in the functioning of the reproductive system and the development of the embryo. Mio-inositol is an important synergist of folates and other vitamins (B5, PP) [1].

The complex of placental regulatory peptides has an immunotropic effect: stimulates the functional capacity of phagocytes of the mucous membranes and blood, increases the synthesis of anti-inflammatory cytokines, and affects the activity of regulatory subpopulations of lymphocytes. With autoimmune diseases or syndromes, it reduces the activity of the immune-dependent inflammatory process, increases the number of CD4 + / 25 + and CD8 + / 25 + cells, especially the level of IL-10 in blood plasma. The complex of placental regulatory peptides has a pronounced anti-inflammatory and spasticity effect, reduces the intensity of destructive, infiltrative and proliferative processes in the inflammatory site, reduces the severity of edema, accelerates the processes of epithelization, regeneration, prevents the development of the adhesion process, the effect on the proliferative and exudative phases of inflammation significantly exceeds the placenta extract [4].

L-arginine aspartate has a positive effect on the expression of markers of angiogenesis, thereby participating in the formation of new blood vessels in the endometrium. In this case, the enhanced formation of nitric oxide leads to dilatation of peripheral vessels and a decrease in the overall peripheral vascular resistance, which contributes to lowering blood pressure and reducing tissue hypoxia. Increasing the enzyme activity of NO- synthase against the background of oral administration of the solution of L-arginine aspartate provides a constant basic level of nitric oxide, which in turn contributes to increasing the density of functional vessels of the endometrium, enhancing its blood supply [2].

Conclusions

Conducting the developed preparation of endometrium for the transfer of vitrified / warmed embryos by the developed method contributes to the continuation of pregnancy and increase the number of live births.

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