Embryological indicators in cycles with HCG or different doses of GnRH-a for the final oocyte maturation in IVF-ICSI patients

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**Abstract**

Application of gonadotropin-releasing hormone agonists (GnRH-a) as a trigger for final oocyte maturation in cycles of in vitro fertilization (IVF) substantially decreases the risk of ovarian hyperstimulation syndrome (OHSS). So far there is no consensus on the optimal dosage of GnRH-a when using it as a trigger in IVF cycles. We compared embryological characteristics in IVF-ICSI cycles when applying triptorelin at a dose of 0,2 mg (test group 2), 0,5 mg (test group 3) and human chorionic gonadotropin (HCG) at a dose of 10,000 IU (test group 1). In group 1, the average number of oocytes per oocyte retrieval (11,7±4,8) was lower in comparison with groups 2 and 3, which can be explained by the differences in the selection of the patients’. The number of oocytes per retrieval in group 3 (20,2±6,3) was significantly higher (p=0.02) compared to group 2 (17,0±6,2). The percentage of mature oocytes (MII) and fertilization rate did not differ between the groups. The rate of blastocyst formation in group 3 (71,9±17,1%) was significantly higher (p=0.02) in comparison with group 2 (57,9±24%). We did not found any significant difference in the rate of embryo implantation in cycles with oocyte donation when HCG or various doses of triptorelin were used as a trigger.

We conclude that application of triptorelin at a dose of 0,5 mg may be more effective for triggering final oocyte maturation in IVF cycles in comparison with the dose of 0,2 mg, due to the increase in the number of retrieved oocytes and the improved rate of the blastocyst formation.

***Introduction***

One of the most frequent and dangerous complications of controlled ovarian stimulation (COS) is the ovarian hyperstimulation syndrome (OHSS), which occurs at the rate of about 3,6-7,5% [1]. Prevention of this complication is a high-priority goal in the optimization of modern assisted reproductive technologies. Reducing the dosage of follicle stimulating hormone (FSH), application of the so-called ‘mild‘ protocols of COS and application of protocols that use antagonists of gonadotropin-releasing hormone (GnRH-ant) can reduce the risk, but cannot completely prevent the development of OHSS. Fundamental research on application of agonists of gonadotropin-releasing hormone (GnRH-a) as an ovulation trigger in the natural cycle was performed in the 1970-s [2]. However, pituitary desensitization in ‘long’ GnRH-a protocols excluded the possibility of using this trigger for the final oocyte maturation. After introduction of GnRH-ant into clinical practice, it was discovered in the early 1990-s that application of GnRH-a as a trigger for the final oocyte maturation results in obtaining mature oocytes and minimizes the risk of OHSS [3]. This method carried certain restrictions: it could be only used in protocols with GnRH-ant and “fresh” embryo transfers, and pronounced insufficiency of the luteal phase resulted in a critically low pregnancy rate [4]. This problem does not arise in the case of oocyte donation or when all embryos get frozen without embryo transfer (‘Freeze all’ method) because there is no negative effect of GnRH-a on the luteal phase and endometrium receptivity [5].

Modern methods of preventing OHSS are unanimous about the fact that application of GnRH-a as a trigger is definitely an effective tool [5]; as for the trigger dosage, authors hold different opinions and suggest using dosages from 0,2 to 0,5 mg of GnRH-a [6, 7].

The objective of this research is to compare the embryological indicators in IVF-ICSI cycles after applying human chorionic gonadotropin (10,000 IU) with GnRH-a in dosages of 0,2 and 0,5 mg as a trigger for final oocyte maturation.

***Materials and Methods***

We have retrospectively analyzed 345 IVF cycles with autologous oocytes carried out at our clinic during 2014. The research included only the cycles with women aged below 35 years, where the number of oocytes per aspyration exceeded five. Follicle growth was stimulated with recombinant and menopausal FSH. In all the COS cycles, the block of the premature luteinizing hormone peak was achieved with GnRH-ant. Indications for prescribing GnRH-a as a trigger were high levels of estradiol on the day the trigger was prescribed (over 3,500 pg/ml) and growth of more than 18 follicles. Before September, 2014 we used a-GnRH as a trigger of final oocyte maturation at a dose of 0,2 mg, from October to December 2014 we used GnRH-a at a dose of 0,5 mg. In all the cases we used ICSI as the method of fertilization.

The cycles selected for the research were divided in 3 groups. The first group contained 252 cycles for which HCG at a dose of 10,000 IU was used as a trigger for the final oocyte maturation. The second group contained 72 cycles for which 0,2 mg of GnRH-a (triptorelin) was used as a trigger. The third group contained 24 cycles for which 0,5 mg of triptorelin was used as a trigger.

The following embryological indicators were recorded: the number of oocytes, oocyte percentage on MII stage, rate of normal (2 PN) and abnormal (1 and 3 PN) fertilization, percentage of embryos with 4 blastomeres 44 hours after fertilization and blastulation rate.

To compare the implantation potential of embryos obtained with HCG or GnRH-a, we additionally selected and analyzed 140 IVF-ICSI cycles with oocyte donation. The group with HCG as a trigger contained 62 cycles, while the groups with triptorelin at a dose of 0,2 mg and 0,5 mg contained 64 and 14 cycles respectively. One or two embryos were transferred into recipients’ uterine cavity on the 3rd or 5th day after fertilization. Clinical pregnancies were verified in the 7th week of gestation, by identifying one or two gestation sacs with confirmed heartbeat during ultrasonography.

*Statistical Analysis*

For statistical analysis we used package SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). We used Kolmogorov-Smirnov test to compare the distribution of the sample data to the normal distribution. When comparing results among the groups, we used Kruskal-Wallis test, while for paired comparison we used Mann-Whitney U test. To compare the results of implantation rate, we used Fisher’s exact test. The results are represented as a mean value and a standard deviation or a standard error of the mean (for the rate of abnormal fertilization and implantation rate). The hypothesis of difference between the groups was accepted when the value was p<0.05.

***Results and Discussion***

In the cycles analyzed in the present research we did not observe any cases of severe or moderate OHSS. The patients’ age did not vary substantially among the test groups and was 30,3±3,31, 30,2±3,09 and 30±2,6 in groups 1, 2 and 3 respectively.

The average number of oocytes per retrieval in group 1 was 11,7±4,8. This characteristic was significantly lower than in the groups with GnRH-a as a trigger, which is accounted for by the influence of patients’ selection (Fig. 1). Groups 2 and 3 predominantly consisted of patients with a high risk of OHSS development and a relatively large number of growing follicles, which resulted in the increase of the average number of oocytes per retrieval in these groups. In groups 2 and 3 we on the average obtained 17±6,2 and 20,2±6,3 oocytes, respectively. It is of particular interest that in group 3 with a higher dosage of triptorelin, the average number of oocytes was significantly higher than in group 2 (p = 0,02).

A similar tendency was observed in the number of obtained mature oocytes: in groups 2 and 3 this characteristic was 12,6±5,8 and 14,6±3,6 respectively, which was significantly higher in comparison with group 1 (8,5±3,9).

As for the percentage of mature oocytes, no statistically significant differences among the test groups were registered (group 1 – 74,4±20,6%, group 2 – 73,6±19,8%, group 3 – 71,3±20,1). This indicates that despite the differences in the absolute number of oocytes per retrieval, the efficacy of final oocyte maturation was on the same level in the HCG group and the groups with different dosages of GnRH-a. Thus, application of triptorelin in both analyzed dosages does not result in decrease of oocyte nuclear maturation in comparison with HCG cycles. At the same time, applying 0,5 mg of triptorelin allows to obtain a greater number of oocytes.



Fig.1. The average number of oocytes per retrieval when using HCG (1), and triptorelin at a dose of 0,2 mg (2) or 0,5 mg (3) as a trigger.

\* – lower than in groups 1 and 2 (p<0.05)

# – higher than in group 2 (p=0.02)

The percentage of the overall number of oocytes that reached the MII stage shows how effectively the trigger initiates nuclear maturation of the oocytes. To evaluate the cytoplasmic maturation efficacy we analyze the rate of normal and abnormal fertilization and characterize the embryos at the second and fifth day of culturing.

We compared the rate of normal fertilization after ICSI among the test groups. The rate of normal fertilization in group 1 was 75,7±16%, which was similar to the results in groups 2 and 3 (75,0±16% and 78,3±11,8% respectively). The rate of normal fertilization is an indirect indicator of oocyte cytoplasmic maturity and the ability of oocytes to regulate the process of normal fertilization. Our results indicate that applying GnRH-a as a trigger does not decrease efficacy of oocyte cytoplasmic maturation and does not influence the rate of normal fertilization. The dosage of triptorelin did not significantly influence the rate of normal fertilization either.

The rate of abnormal fertilization can also serve as an additional characteristic of the quality of the obtained oocytes [8]. In the present research, the rate of zygote formation with 1PN was 0,9±0,2% in the HCG group, 1,5±0,43% in test group 2 and 0,5±0,4% in test group 3. The rate of 3PN zygote formation was 2,0±0,4%, 2,3±0,5% and 1,8±0,7% in groups 1, 2 and 3 respectively. Rates of abnormal fertilization in all test groups did not exceed results published by other authors [9, 10]. Of particular interest is the fact that in group 2 rate of fertilization with zygotes 1PN and 3PN was somewhat higher than in group 1 (p=0.027 и p=0,021 respectively) and did not differ from group 3. Though in view of the low occurrence rate of the abnormal fertilization the significance of differences between groups 1 and 2 is debatable, the increase in the rate of abnormal fertilization may indicate decreased cytoplasmic competence of oocytes obtained [11]. At the same time, according to previous publications, patients with good and excessive response produce a greater number of 3PN zygotes [12].

It is accepted that embryo development in the first 3 days after fertilization is significantly determined by the mother’s factors introduced by the oocyte. The number of blastomeres in embryos at predefined points in time is an indicator of their quality. In the present research we compared the rates of obtaining embryos with 4 blastomeres estimated 44 hours after fertilization. This characteristic did not significantly differ among the groups and was 63,1±26,8%, 61,3±24,9% and 68,7±17,1% in groups 1, 2 and 3 respectively. These results are in line with our data on the rates of normal fertilization.

Capability of embryos to develop to the blastocyst stage is a strong predictor of implantation and IVF cycle effectiveness [13]. At the same time, oocyte competence in IVF cycles substantially determines an embryo’s ability to develop to the blastocyst stage [14]. The rate of blastulation in groups 1, 2 and 3 was 66,6±30,3%, 57,9±24% and 71,9±17,1% respectively (Fig. 2). Notably, the rate of the blastocyst formation was significantly higher in the group taking 0,5 mg of triptorelin than in the group taking 0,2 mg (p=0,02).

Thus, in the IVF cycles with the trigger GnRH-a at a dose of 0,5 mg the number of oocytes per retrieval and the rate of blastulation were higher in comparison with the cycles with the trigger at a dose of 0,2 mg. We can hypothesize that the observed effect is related to the increase in the amplitude of the endogenous FSH surge when a higher dose of GnRH-a trigger is used, which possibly creates more physiological conditions for oocyte maturation. This assumption is indirectly corroborated by the research in the field of *in vitro maturation* which shows that presence and concentration of FSH in the culture medium are of significant importance at the stage of the final maturation of oocytes [15].

At the final stage of the research we wanted to check if the kind and dosage of the trigger applied influences the implantation potential of embryos. It is known that applying GnRH-a as a trigger of the final oocyte maturation is connected with a more pronounced defect of the lutein phase than in HCG-triggered cycles. That, in turn, negatively influences the endometrium receptivity and the efficacy of fresh cycles with GnRH-a trigger [4]. Application of GnRH-a trigger in donor cycles is devoid of this problem because recipients are not exposed to the influence of COS factors.

Taking this into consideration, we compared the implantation rate of the embryos only in cycles with oocyte donation, for which HCG or triptorelin at a dose of 0,2 or 0,5 mg were used as a trigger. The implantation rate of the embryos obtained using donors’ oocytes was 41,9±2,4%, 42,1±4,7% and 40,9±8,5% in groups 1, 2 and 3 respectively. We conclude that neither the kind, nor the dosage of the trigger applied made significant influence on the ability of the embryos to implant.



Fig. 2. Rate of blastocyst formation when using HCG or triptorelin at a dose of 0,2 or 0,5 mg as a trigger.

# – higher than in group 2 (p=0.019)

***Conclusion***

In the groups where triptorelin was used to trigger final oocyte maturation, embryological characteristics were on the same level as with the HCG-triggered cycles. This confirms that the application of GnRH-a as a trigger in IVF cycles is no less effective than application of the classical HCG trigger. In addition, we found that in the IVF cycles for which the GnRH-a trigger was used at a dose of 0,5 mg, the number of oocytes per retrieval and the blastulation rate were higher than in the cycles with the same trigger at a dose of 0,2 mg. If this effect is confirmed in further research, the application of triptorelin at a dose of 0,5 mg as a trigger of final oocyte maturation could be widely recommended, to increase the cumulative efficacy of IVF cycles with high and excessive response to COS.

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