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ORIGINAL ARTICLE

Analysis of factors influencing morphokinetic characteristics of embryos in ART cycles

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Abstract

In this article, some factors were evaluated for their impact on embryo morphokinetics during assisted reproductive technology (ART) cycles. We detected significant differences in the fourth cell division time (t5) of embryos obtained after controlled ovarian stimulation in long GnRH agonists and GnRH antagonist protocols. We also found that higher gonadotropin dose may slow down the development of embryos. However, both male and female age, the number of oocytes and number of normal forms of sperm in the ejaculate did not affect the kinetic parameters of embryo development. Further research is needed to identify all the spectrum of factors, which can affect the rate of embryo development.

Keywords

COS protocol, embryo, FSH, implantation, IVF, morphokinetics, time-lapse

History

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Introduction

One of the central problems of clinical embryology is the choice of an embryo with the highest implantation potential for transfer to the uterus. Recently, a number of innovative approaches such as evaluation of metabolic characteristics [1], transcriptional profile [2] and genetic competence of embryos [3] have been developed to address this problem. The method based on the analysis of morphokinetic parameters of the developing embryo attracts special attention [4].

One of the problems that makes wide application of morphokinetic embryo selection complicated is the fact that embryo cleavage rates vary in response to the influence of different factors. Thus, it was shown that polycystic ovary syndrome [5], culture medium [6], culturing conditions [7], smoking [8] as well as some other factors influence embryo morphokinetics. At the same time a wide range of factors still remains unexplored with this respect. We suggested that the conditions determining efficiency of assisted reproductive technology (ART) cycles could also affect the dynamics of embryo development. Thus, the aim of this work was to study the influence of different factors on morphokinetic parameters of embryos in ART cycles.

Materials and methods

The data used in this research were obtained after informed consent of patients in appliance with ethical guidelines during the IVF cycles, which were carried out in Academician V.I. Gryshchenko Clinic for Reproductive Medicine within the period from June 2012 to April 2014. The range of women's age was 23–49. All the embryos were obtained after fertilization with intra-cytoplasmic sperm injection (ICSI). The embryo transfers were carried out on the 3rd or the 5th day after fertilization. The implantation of the transferred embryos was checked after 7 weeks of pregnancy at identification of gestation sac with confirmed heartbeat during ultrasonography.

The embryos were cultured in Thermo Heracell 150i incubators at temperature 37 °C in the atmosphere with N_2, CO_2 and O_2 contents of 89-, 6- and 5%, respectively. The culturing of embryos was carried out in COOK media in special dishes for group culturing (Vitrolife, Hungary) with 9 or 16 microwells. The system Primovision (Vitrolife, Hungary) which was adjusted on monitoring frequency of 10 min was used for time-lapse monitoring. The exact times of cell events after fertilization with ICSI were annotated manually in software Primovision Analyzer at viewing videoloops of each embryo. The following morphokinetic parameters of embryos were used: the cleavage time to five (t5), four (t4), three (t3) and two (t2) blastomeres, duration of second cell cycle (cc2) and the time between t5 and t3 (cc3). The normal ranges for these parameters were adopted in accordance with Meseguer et al. [9]. The statistical analysis was carried out by comparison of average values of time of cell events by means of Student test.

Results and discussion

One hundred and thirty ICSI cycles with consequent 127 transfers of embryos were performed with time-lapse monitoring (15 of which were with oocyte donation). The average number of embryos per transfer was 2.06. Morphological criteria were considered to be primary for embryo choice but if embryos were morphologically equal then their kinetic parameters were assessed and used for selection. Ninety two of the 262 embryos transferred have been implanted with corresponding implantation rate of 35.1%.

It is known that female age is one of the essential factors influencing effectiveness of IVF cycles [10,11]. This influence is caused by increased aneuploidy rate and other genetical

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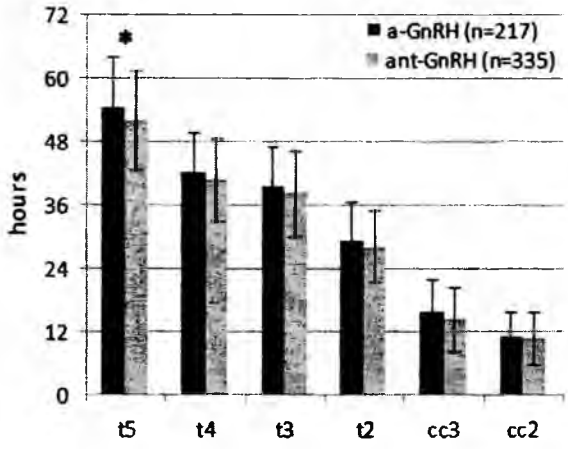

Figure 1. Timing of embryo events (h) in ART cycles with the use of GnRH-a and GnRH-ant protocols. *–difference between groups is significant ($p = 0.039$).

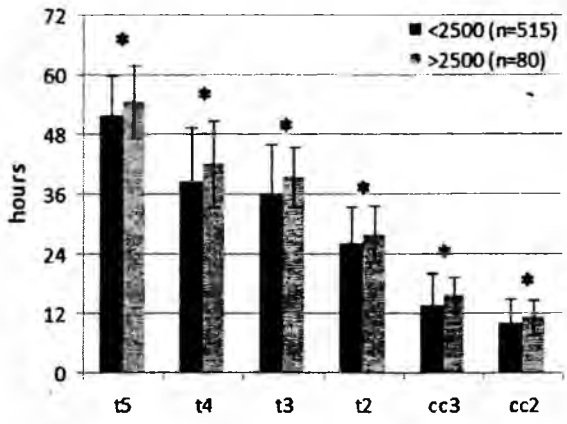

Figure 2. Timing of embryo events (h) in ART cycles with various total FSH doses (value for separation of groups is 2500 IU). *–differences between groups are significant ($p < 0.05$).

abnormalities in oocytes of age patients [12,13]. Advanced female age is also associated with aging of mitochondrial apparatus and reduced oocyte ability to repair fragmented DNA [14]. We proposed that oocyte aging could also affect the rates of embryo cleavage. To test this hypothesis, we compared morphokinetic parameters of embryos obtained in women younger ($n = 530$) and older than 40 ($n = 86$). The estimated values did not differ between the studied groups essentially (data not shown). The average values of morphokinetic parameters in both groups corresponded to normal ranges.

The role of male age on ART success has also been actively investigated. There were data showing that miscarriage rates increase in cycles with the age of the partner above 50 [15,16]. In another study, the influence of male age on ART effectiveness was not confirmed [17]. In our work, morphokinetic characteristics of the embryos have been compared in the cycles where the age of men was more ($n = 58$) or less than 50 ($n = 606$). Evaluated parameters have not been found to be statistically different between the groups, their average values appear to be within the limits of normal ranges (data not shown).

The choice of controlled ovarian stimulation (COS) protocol depends on many clinical factors and in many aspects determines efficiency of ART cycles. The most wide-spread stimulation protocols are a long protocol with gonadotropin-releasing hormone agonists (GnRH-a) and a protocol with GnRH antagonists (GnRH-ant). In this article, we carried out the comparison of morphokinetic parameters of the embryos obtained in cycles with GnRH-a and GnRH-ant. It was found that average time of the fourth cell division in the group of embryos with a long protocol (54 h 25 min) statistically exceeds this value in the group with the antagonist protocol (52 h 36 min; Figure 1). It should be noted that the average values of t5 time in both groups were inside the normal range of 48–56 h. These results corroborate earlier results [18], where it was also shown that the embryos obtained in protocols with agonists developed more slowly than in GnRH-ant protocols.

It was shown earlier, that application of total FSH dose above 2200 IU during COS was associated with reduction in pregnancy rate [19]. In this article, the assumption was made that the rates of embryo cleavage might depend on the total dose of FSH used for COS. In order to test this hypothesis, we carried out the comparison of morphokinetic parameters of embryos from the groups with total FSH dose lower and higher than 1400, 1800 and 2500 IU. We used three couples of comparison groups in order to determine more precisely at which total FSH dose the influence of this factor is pronounced at most.

The average values of morphokinetic parameters in the groups obtained at threshold values of the total FSH dose of 1400 and 1800 IU did not show differences among themselves (data not shown). At the same time, groups formed with threshold value of 2500 IU, differed significantly among themselves on all morphokinetic parameters evaluated (Figure 2). The difference between groups was about 3 h on t5, t4, t3 parameters and about 2 h on t2 time. The interval characteristics cc3 and cc2 differed between groups for 2 h and 80 minutes, respectively. It should be noted, that the use of total FSH dose more than 2500 IU was accompanied by prolongation of studied time parameters. These results correspond to the data of Munoz et al. [20], at the same time prolongation of embryo development was also observed at reduced total FSH dose in the group of patients with deficiency of body mass [21]. It can be explained by the presence of non-linear dependence between the rate of embryo development and the total FSH dose obtained. On the other hand, the usage of higher or lower total FSH doses depends on particular clinical group of patients. Hence, it is also impossible to exclude that decrease of rates of embryo development in the group with increased total FSH dose is due to biological peculiarities of representatives of this group.

The important criterion of male fertility is the content of normal forms of spermatozoa in ejaculate. It was found that the abnormal morphology of spermatozoa can be associated with a number of epigenetic defects such as failures of DNA methylation [22], defects of the centrosome [23], increased fragmentation of spermatozoa chromatin [24]. We proposed that these epigenetic defects as well as some other factors related to the morphology of spermatozoa could affect the rate of embryo development. Morphokinetic parameters of embryos were compared between the cycles with higher ($n = 425$) and lower ($n = 170$) than 4% content of normal forms of sperms in ejaculate (threshold value according to the 5-th manual of the WHO). As a result, no differences were found on studied characteristics between two groups (data not shown). Average values of all parameters were within the limits of normal ranges.

It was shown that the efficiency of ART cycles grows linearly together with increase of the amount of oocytes up to a certain threshold value. At number of oocytes increasing more than 15 per cycle the efficiency of ART cycles stops growing [25]. At the same time, presence of 14 antral follicles at the moment of prescription of the trigger of final maturing of oocytes is a

boundary criterion for prediction of the hyper-response on COS [26]. In this article, we questioned possible influence of oocyte number on morphokinetic parameters of embryos. Two groups of comparison were separated with a threshold value of 15 oocytes. Morphokinetic characteristics of embryos obtained in cycles where the number of oocytes was 15 and less ($n = 352$) did not differ from the group with more than 15 oocytes ($n = 298$) and were within the limits of normal ranges (data not shown).

Thus, we demonstrated that total FSH dose and COS protocol influence the kinetics of embryo development. At the same time the age of partners, the amount of oocytes and the content of normal forms of spermatozoa in ejaculate had no effect on studied morphokinetic parameters of embryos. Further research is necessary to reveal the factors that influence the rate of embryo development. Based on these data, the flexible morphokinetic model should be developed for accurate prediction of embryo viability.

Declaration of interest

The authors have no conflicts of interest relevant to this article.

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