ORIGINAL ARTICLE

Influence of female age on blastulation rate of embryo produced by ICSI

Influência da idade da mulher na taxa de blastulação de embriões produzidos por ICSI*

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ABSTRACT

OBJECTIVES: There is a tendency to adopt prolonged culture in older patients; however there are no conclusive results about the influence of age on blastulation rate. Therefore, we decided to analyze the influence of female age on prolonged culture results. **METHODS:** One hundred and seven ICSI procedures performed in our center from January 1999 to December 2000 were retrospectively analyzed. The blastulation rate was verified and correlated with patient age. **RESULTS:** In average, 2.8 blastocysts/patient were transferred. The blastulation rate for each age group was: 180 (32%) in the group < 35 years; 163 (29%) in the group 35-40 years, and 118 (21%) in the group > 40 years. The statistical analysis demonstrated a significant difference ($p < 0.05$). **CONCLUSION:** The percentage of embryos that achieved the blastocyst stage was different in each age group and this percentage dropped as patient age increased. Female age may influence on blastulation rate of pre-embryos, observing a drop in this rate as patient age increased.

Keywords: Embryo; Blastocyst; Maternal age; Sperm-injections, intracytoplasmic; Reproduction

RESUMO

OBJETIVOS: Existe uma tendência em se adotar a cultura prolongada para pacientes mais idosas, porém não se têm ainda resultados conclusivos sobre a influência da idade sobre o índice de blastulação. Portanto, decidimos realizar uma análise da influência da idade da mulher nos resultados da cultura prolongada. **MÉTODOS:** Foram avaliados retrospectivamente 107 procedimentos de ICSI realizados no nosso centro no período de janeiro/1999 a dezembro/2000. Foi verificada a taxa de blastulação e foi feita a relação dessa taxa com a idade da paciente. **RESULTADOS:** Foram transferidos em média 2,8 blastocistos por paciente. A taxa de blastulação encontrada em cada faixa etária foi: 180 (32%) para pacientes com menos de 35 anos; 163 (29%) para o grupo entre 35 e 40 anos e 118 (21%) para o grupo de pacientes com mais de 40 anos. De acordo com a análise estatística esses dados possuem diferença significativa ($p < 0,05$). **CONCLUSÃO:** A porcentagem de embriões que alcançaram o estágio de blastocisto mostrou-se diferente para cada faixa etária, sendo que a porcentagem de embriões que chegaram ao estágio de blastocisto diminuiu conforme a idade da paciente aumentou. A idade da mulher pode influenciar no índice de blastulação dos pré-embriões, observando-se decréscimo desse índice com o aumento da idade da paciente.

Descritores: Embrião; Blastocisto; Idade materna; Injeções de esperma intracitoplásmicas; Reprodução

INTRODUCTION

At present women initiate their families at more advanced age due to several social reasons. However, it is known that maternal age is still an important limiting factor for older couples to get pregnant(1). A study was carried out and verified that oocytes of older patients presented a higher incidence of aneuploidy(2). Another study demonstrated that oocytes of older donors had a higher incidence of abnormalities in meiotic spindle(3).

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An alternative found to select the best pre-embryos for transfer is the use of prolonged culture. It is believed that only the best pre-embryos are able to achieve this stage leading to greater selection. A study was conducted comparing pregnancy and implantation rates of pre-embryos transferred at day 3 or 5 after follicular aspiration. They found implantation and pregnancy rates higher in patients in whom the pre-embryos were transferred at day 5[4]. Thus, according to these authors, blastocyst transfer (day 5) would help implantation, and allow to use less pre-embryos not affecting pregnancy rate, as compared to pre-embryo transfer at day 3[5-6].

However, there is a concern about adopting this method for older patients, since there are no conclusive results about the influence of maternal age on blastulation rate. Hence we decided to carry out an analysis of the influence of female age on the results of prolonged embryo culture in our center.

METHODS

One hundred and seven intracytoplasmic sperm injection (ICSI) procedures performed in the Human Reproduction Unit at Hospital Israelita Albert Einstein, from January 1999 to December 2000 were assessed. The statistical analysis was performed by c^2 test.

An ovarian blockade with a luteinizing hormone-releasing hormone (LHRH) agonist was performed, followed by controlled ovarian hyperstimulation induced by administration of human menopausal gonadotropin, urofollitropin (FSH) or recombinant FSH. When the follicle achieved a maximum diameter of 20-22 mm, human chorionic gonadotropin (hCG) was injected intramuscularly. The follicles were aspirated 34-36 hours after administration of hCG by transvaginal ultrasound. Micromanipulation was performed in plastic Petri dishes (Falcon, USA), in 5ml microdrops of modified HTF medium (Irvine Scientific, USA), supplemented with human serum albumin 5% (Irvine Scientific, USA)[7]. Fertilization was observed 17-21 hours after injection and the morphological quality of pre-embryos was determined according to the morphological criteria established by Veeck[8]. The semen samples were obtained by masturbation and processing was conducted by discontinuing gradient centrifugation (Isolate - Irvine Scientific cod 99257, 99258). The pre-embryos sent to prolonged culture were kept up to day 3 in HTF medium supplemented with synthetic serum substitute (SSS) 10%. In day 3 they were put in a specific blastocyst culture medium and enriched with SSS 10%. The blastulation rate was determined and correlated with patient age. Transfer or freezing was performed between days 5 and 7 of development.

RESULTS

The sample analyzed consisted of 107 patients distributed as follows: 70 (65.42%) aged < 35 years, 33 (30.84%) aged 35-40 years and only 4 (3.74%) aged > 40 years.

We selected 562 pre-embryos for blastocyst representing 50% of the total number of formed pre-embryos. In average, 2.8 blastocysts/patient were transferred. The blastulation rate found for each age group was 180 (32%) in the group aged < 35 years, 163 (29%) in the 35-40- year group, and 118 (21%) in those aged > 40 years. The statistical analysis of these data shows a significant difference ($p < 0.05$), demonstrating the percentage of embryos that achieved the blastocyst stage is different in each age group; and this percentage drops as patient age increases (table 1).

As to number of clinical gestations (n = 30), 22 (73.33%) were in the group aged < 35 years and 8 (26.67%) in the group 35-40 years. There was no positive result in the group aged > 40 years.

Table 1: Age group and blastulation

Age group (years)	Embryos	Blastocysts	Percentage
< 35	395	127	32%*a
35-40	153	45	29%*b
> 40	14	3	21%*c
Total	562	175	31%

*a ≠ *b and ≠ *c and *b ≠ *c; *p<0.05

DISCUSSION

The studies reported in the literature on the effect of female age on capacity of pre-embryos developing up to blastocyst stage are not conclusive[4-5]. One of them demonstrated that there is no correlation between maternal age and capacity to form blastocysts[7]. Nevertheless, another author reported reduced capacity of pre-embryos developing up to blastocyst stage as maternal age increases[9]. There were similar findings reporting that female age is related not only to blastulation rate, but also to decreased implantation and pregnancy rates[10].

This study evaluated blastulation rate and age group of patients. We observed a significant drop in this rate; in that, the rate for younger patients aged < 35 years was 32% and for older, aged > 40 years, it was 21%. Our results agree with the data previously published, confirming there might be an influence of patient age on blastulation rate[9-10]. Consequently, in these cases, the results also influence on implantation and pregnancy rates.

We verified that most positive b-hCG tests were collected in patients aged < 35 years (n = 22; 73.33%). There was a marked decrease in clinical pregnancy rate in the group aged 35-40 years (n = 8; 26.67%), as compared with the younger group. There was no positive result in the group aged > 40 years.

This study is valid since it provides information on blastulation rate behavior in relation to female age in the frequently observed situations of prolonged pre-embryo culture. Thus, we concluded the age of woman submitted to ICSI procedure may influence on the blastulation rate of pre-embryos, and there was a drop in this rate as maternal age increased.

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