Human embryo cultivation in melatonin containing medium

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INTRODUCTION

The goal of embryo culture in an assisted reproductive (ART) programme is to improve the quality of embryos developing in the laboratory and the chances of successful delivery of a healthy baby. The main aim of *in vitro*-culture is to reproduce the *in vivo*-conditions, in order to obtain an optimal embryonic development to maximize the outcome.

Oxidative stress appears to be one of the causes of impaired *in vitro* embryo development. The protective role of free radical scavengers in maturation and culture medium has been documented (Tsantarioutou et al., 2007; Kang et al., 2009; Manjunatha et al., 2009). Melatonin acts as a potent scavenger of free radicals (Tan et al., 2007).

Although there are some studies describing the beneficial effect of melatonin on in vitro embryo development in different species (Ishizuka et al., 2000; Papis et al., 2007; Rodriguez-Osorio et al., 2007), most of them report the addition of melatonin in embryo culture medium and in those which study the effect of melatonin during in vitro maturation.

OBJECTIVE

The aim of this study is to compare the effectiveness of Human embryo cultivation in medium with different concentration of melatonin.

MATERIALS AND METHODS

Study Design

The study was carried out in AltraVita IVF Clinic, Moscow. In the present prospective study embryos obtained from 128 couples undergoing ICSI or IMSI Hoffman modulation (Yakovenko S.et al., 2009, Apryshko V. et al., 2010) were distributed between culture media LifeGlobal (total number of embryos – 1074), LifeGlobal + 10^{-9} M Melatonin (total number of embryos – 540) and LifeGlobal + 10^{-6} M Melatonin (total number of embryos – 425). Sibling embryos were randomly assigned to culture in control and melatonin supplemented medium. Those 128 couples aged between 22 and 41 (the average women age was 31.4), were neither suffering from genetic diseases nor bearing cryptozoospermia.

Ovarian stimulation

The induction of superovulation carried out over a short protocol using antGnRG with 2-3-th day of MC using recombinant and / or urinary gonadotropins in a daily dose of 150-300 IU. Ovulation is initiated by the injection of hCG at a dose of 10 000 IU for 34-36 hours prior the oocyte punction.

Oocyte retrieval

Aspirate follicles performed under intravenous anesthesia. The cumulus oocyte complex was obtained by transvaginal follicle punction under ultrasound guidance after 34-36 hours after hCG injection.

Fertilization

Oocytes were fertilized using ICSI or IMSI Hoffman modulation (*Yakovenko S.*et al., 2009, Apryshko V. et al., 2010), depending on semen parameters.

Embryo culture

In the experiment, embryos were cultured under an atmosphere of 7.3% CO2 and 20% O2 at 36.7°C with different concentration (10^{-9} and 10^{-6} M) of melatonin (Sigma, USA) in LifeGlobal culture media. The culture medium was changed on day 3 and embryos were checked briefly every day.

The resulting blastocysts were used either for embryo transfer or cryopreservation.

Embryo morfology evaluation

Zygotes and embryos were individually evaluated after ~ 18, ~ 45, ~ 72 and ~ 96 hours after fertilization. On the night of 5-6 were evaluated quality blastocysts formed. For the embryo transfer (ET) and cryopreservation using expansion blastocyst quality AA, AB, BA and BB by Gardner D.K. (2000).

Statistical analysis

The research results are expressed as average ± SD. Data were compared using Student's t-tests.

RESULTS

The study results are presented in the table below.

TABLE. Results

	LifeGlobal + 10^{-9} M Melatonin (n=540), Mean ± SE	LifeGlobal (control 1) (n=590), Mean ± SE	LifeGlobal + 10^{-6} M Melatonin (n=425), Mean ± SE	LifeGlobal (control2) (n=484), Mean ± SE
4 and more-cells embryos on day 2, %	50.02±3.4	51.13±3.43	59.68±3.45	59.06±3.31
8 and more-cells embryos on day 3, %	40.97±3.53	37.37±3.20	44.05±3.28	44.94±3.46
Compacted embryos on day 4, %	45.36±3.49	43.82±3.58	50.91±3.98	46.23±3.72
Good quality blastocyst on day 5, %	19.82±2.48	17.84±2.11	23.36±2.70	23.04±2.68
Good quality blastocyst on day 6, %	16.43±1.97	16.03±1.88	15.90±2.11	14.78±2.02
Total blastocyst, %	36.25±2.79	33.87±2.52	39.26±3.37	37.82±2.97

CONCLUSIONS

Our study did not reveal statistically significant differences in effectiveness of human embryo cultivation in melatonin or non-melatonin medium. Also, in the present study, pharmacological concentrations of melatonin seem to be non-toxic to human early embryos.

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