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Quality of Intact and Artificially Collapsed Human Blastocysts after Vitrification

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It is well known that the survival rate of embryos after vitrification depends on their expansion stage. We aimed to compare behavior of early (n=31) and expanded blastocysts (n=83) after vitrification. Expanded Day 5 embryos were divided into two groups – artificially collapsed (n=38) and intact (n=45). All blastocysts selected for vitrification were Day 5, classified into three groups – excellent, good and average quality. Thawed blastocysts were cultivated for at least 3 hours before embryo transfer. Assessment of their vitality and re-expansion was conducted every hour. Our study showed that mechanical collapse of blastocoele through micropipette puncture has a positive effect on embryo survival rate. The greatest survival rate (96.8%) and the fastest re-expansion were observed in early blastocysts. 35 (92.1%) survived thawing in the group of deflated blastocysts, while 39 embryos (86.6%) survived in the group of untreated before vitrification.

Key words: blastocyst, vitrification, artificial collapse

Introduction

Embryo cryopreservation has multiple benefits and is a routine procedure in most in vitro fertilization programs. Compared to slow freezing vitrification provides minimal embryo damage and higher survival rates. Cryopreservation prevents discard of surplus embryos and makes it possible to store them for future use [6].

It is well known that the efficiency of blastocyst vitrification depends on the expansion stage of fluid-filled cavity [1, 2, 3, 7]. While expanded blastocysts are more vulnerable to ice formation, early blastocysts have better post thaw survival rate due to the small amount of blastocoeli fluid [5]. For that reason we decided to compare behavior of early and expanded blastocysts after vitrification.

Material and Methods

Couples who took part in this research were patients of Medical Center for Assisted Reproduction – Varna. They were diagnosed with male factor infertility, unexplained infertility or female factor infertility. The age range varied between 27-43 years for men and between 28-39 years for women. All participants in the research provided informed consent.

The semen samples were obtained through masturbation after 3-5 days of ejaculatory. Concentration, motility and morphology were evaluated in accordance with the WHO's 2010 requirements. Ejaculates were prepared by density gradient centrifugation.

To stimulate follicle development, gonadotropin releasing hormone antagonists were administered. Follicle punctures were conducted under short-term anaesthesia and ultrasound guidance. Oocytes were fertilized by conventional in vitro fertilization or intracytoplasmic sperm injection. Embryos were cultivated in culture medium prior to vitrification. Blastocysts behaviour after thawing was assessed according to the degree of their cavity expansion. Early (n=31) and expanded (n=83) blastocysts were examined. Expanded Day 5 embryos were divided into two groups – artificially collapsed (n=38) and intact (n=45). Blastocyst collapse was performed by micropipetting in buffered medium. All blastocysts selected for vitrification were Day 5, and were classified into three groups – excellent, good and average quality.

Only one embryo per cryotop was loaded. After thawing blastocysts were cultivated for at least 3 hours. Assessment of their vitality and re-expansion was conducted every hour. Single embryo transfer was performed in all cases. Blood pregnancy tests were conducted 10 days after embryo replacement. Pregnancy confirmation ultrasound scan was performed two weeks later.

Results

Table 1 reveals the outcomes after thawing of early, expanded and artificially collapsed blastocysts. The greatest survival rate (96.8%, 30/31) and the fastest re-expansion were observed in early blastocysts ($p=0.01$). The survival rate in artificially collapsed and intact Day 5 embryos after thawing was 92.1% (35/38) and 86.6% (39/45) respectively. We didn't notice any significant difference in time needed for blastocoele re-expansion between artificially collapsed and intact Day 5 embryos. Blastocysts which failed to re-expand were 7.9% (3/38) in the deflated group and 13.34% (6/45) in the group of untreated expanded blastocysts ($p<0.001$). Clinical pregnancy rate did not differ between the three groups.

We do not report miscarriage rate and live birth rate because most of the pregnancies are still ongoing.

Table 1. Comparison of outcomes after thawing of early, expanded and artificially collapsed blastocysts.

	Early blastocysts n (%)	**Artificially collapsed blastocysts n (%)**	**Intact expanded blastocysts n (%)**
Embryos	31	38	45
IVF	9 (29,03%)	11 (28.95%)	10 (22.22%)
ICSI	22 (70,97%)	27 (71.05)	35 (77.78)
Survival rate after thawing	30 (96.8%)	35 (92.1%)	39 (86.6%)
1st hour re-expansion,	21 (67.74%)	17 (44.74%)	19 (42.22%)
2nd hour re-expansion	10 (32.26%)	17 (44.74%)	20 (44.44%)
3rd hour re-expansion	0 (0%)	1 (2.62%)	0 (0%)
lack of re-expansion	0 (0%)	3 (7.9%)	6 (13.34%)
Clinical pregnancy rate	14 (45.16%)	17 (44.74%)	20 (44.44%)

Discussion

The blastocyst expansion stage affects embryo quality after vitrification. Unlike early D5 embryos, expanded blastocysts contain a greater amount of liquid. This prolongs their exposure in cryoprotectants and increases the risk of ice crystals formation.

In this study it was found that early blastocysts had the highest survival rate after thawing. When blastocysts were fully expanded artificial collapse before freezing improved their chances of survival. Lower survival rate in the group of intact expanded blastocysts can be attributed to the fact that excessive amount of water led to the formation of ice crystals and embryo damage.

Raju et al. also reported that compared to deflated expanded blastocysts early blastocysts have better survival rate after thawing [5].

Our findings lend further support to the proposition by Mitsuhata et al. [4] that artificially collapsed blastocysts have a significantly higher survival rate when compared to those which did not undergo microsuction. However in contrast to us, Mitsuhata et al. [4] also reported higher implantation and live birth rates in the deflated group than in the group of intact expanded blastocysts. But according to them these differences were not statistically significant [4].

We didn't find any difference in clinical pregnancy rate between the three groups but this can be attributed to the small sample size of our research.

We can conclude that artificial collapse by micropipetting before vitrification benefits the survival rate of expanded blastocysts.

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