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Live birth potential of good morphology and vitrified blastocysts presenting abnormal cell divisions

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ABSTRACT

This study included 238 good morphology blastocysts, which were transferred after vitrification-warming to 152 women by single blastocyst transfer in Holbæk Fertility Clinic, Denmark. Time-lapse recordings of transferred good morphology blastocysts were reassessed to recognize every abnormal cell division (ACD) from the 1st to the 4th cell cycle. ACDs were distinguished as failed cell divisions and multi-cell divisions.

ACDs were recognized in 37.0% (no. 88/238) of good morphology blastocysts that were vitrified-warmed and transferred in our clinic. Good morphology blastocysts with ACDs showed a lower live birth rate (17.0%) than blastocyst with solely regular cell divisions (29.3%). ACDs could occur at more than one cell division in the same good morphology blastocyst. Reported as independent events, we observed ACDs occurring more frequently at the later cell cycles (1st: 1.3%; 2nd: 8.0%; 3rd: 18.5%; 4th: 18.1%). More blastocysts presented failed cell divisions (no. 95) than multi-cell divisions (no. 14). Live births were achieved from blastocysts showing multi-cell divisions at any cell cycle and failed cell divisions from the 2nd cell cycle. Analyses of the subgroup of first blastocyst transferred to each patient showed similar to results.

In conclusion, good morphology blastocysts presenting ACDs can result in live birth although lower compared to blastocysts with solely regular cell division. Pre-implantation embryos in vitro may undergo self-selection or correcting processes. This supports the transfer of blastocysts instead of cleavage stage embryos, giving first priority to blastocyst showing solely regular cell divisions, and giving second priority to blastocysts presenting ACDs at any cell cycle.

1. Introduction

Recent progresses in embryo culture have increased the interest in blastocysts, due to their higher delivery rate per transfer [1,2]. The introduction of single-step media, which has proved to be just as safe and effective as multistep media [3,4], has reduced the workflow in the laboratory. Moreover time-lapse setup has made embryo scoring more flexible since based on recorded videos [5].

Time-lapse setup has provided also a larger amount of morphokinetics details about early embryo development. Many studies have proposed new scoring models based on these information [5–8]. However no consensus as been reached [9,10].

Among these morphokinetic events, direct cleavage, i.e. multi-cell divisions at the first two cell cycles, have attracted lots of attention, since embryos showing this abnormalities have been reported to have low implantation rate [11,12]. Nevertheless, embryos presenting abnormal cell divisions (ACDs) could develop to blastocyst [13–15]. A recent study has reported 3 live births from blastocysts showing abnormal 2nd cell cycle, i.e., from 2 to 5 cells [16].

The aim of this study was to investigate the occurrence of ACDs in embryos developing to good morphology blastocysts and transferred in our clinic. Furthermore, we evaluated the impact of ACDs on the live birth potential of these blastocysts.

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2. Material and methods

2.1. Study population

This retrospective cohort study included 152 couples undergoing ICSI treatment at Holbaek Fertility Clinic (Holbaek Hospital, Denmark) between 1 January 2013 and 31 May 2015. All women had a BMI between 18 and 35 kg/m2 and were aged <40 years old. All women had regular menstruation, and normal ovaries detected by ultrasound. Only males with minimum 1 million motile spermatozoa per ejaculate were included.

The National Ethical Committee of Medical Science of Denmark approved the study. Informed consent was obtained from all individual participants included in the study.

2.2. Ovarian stimulation

Ovarian stimulation was performed with recombinant follicular-stimulating hormone (Gonal-F®, Merck Serono, Denmark or Puregon®, Organon, Denmark) using an agonist protocol [17]. Follicle growth was monitored by an ultrasound examination, and recombinant hCG (rhCG) (Ovitrelle®, Merck Serono) was given when at least three follicles reached a diameter of 17 mm. Oocyte aspiration was performed 36 h after rhCG administration.

Transfers on day 2 were supported by progesterone (Crinone©, Watson Pharma, Denmark) administration. Transfers of blastocysts were supported by administration of oestradiol (Estrofem®, Novo Nordisk, Denmark) from cycle day 2 for 11–14 days. When the endometrium reached 7 mm or more, progesterone support (Crinone) was given for five days before blastocyst transfer.

A serum beta-hCG test was performed 11 days after blastocyst transfer. Clinical pregnancy was confirmed by transvaginal ultrasound 3–4 weeks after a positive serum-hCG. All clinical pregnancies with a live foetus were followed to delivery. Live birth was defined as a live born baby after gestational age of 24 weeks.

2.3. Fertilization and embryo culture

Cumulus–oocyte complexes were washed and cultured for 2 h in G-IVF™ plus (Vitrolife, Sweden), and the cumulus cells were consecutively removed by hyaluronidase treatment [18]. The denuded oocytes were placed individually in droplets of TL medium™ (Vitrolife) covered by mineral oil [18]. The metaphase II oocytes immediately underwent ICSI and were cultured individually in single-step medium (TL medium, Vitrolife) and an atmosphere of 5.0% O2, 5.5% CO2, and 89.5% N2, controlled by the time-lapse incubator, EmbryoScope™ (Unisense, Sweden).

2.4. Embryo selection for transfer

One or two embryos were eventually selected and transferred on day 2. The number of embryos transferred was based on clinical history of patients. Five operators performed embryo selection for transfer by conventional scoring at the three pre-set times (27): At 18 h post insemination (PI) the fertilization, e.g., presence of two pronuclei (2PN) and the 2nd polar body was assessed; At 25 h PI early cell division defined as the presence of two blastomeres was assessed. Finally at 45 h PI the fragmentation rate and the number of blastomeres were scored. Only embryos with 2nd polar body, 2PN, and at least the first cell division completed were selected for transfer.

Surplus embryos with 2nd polar body and 2 PN were cultured to day 5. The resulting blastocysts were assessed at 116–120 h PI according to Gardner [19] and vitrified for later transfer. The minimum morphological requirements for vitrification were: i) Expanded blastocoel (minimum grade 4); ii) Good inner cell mass (ICM) (grade A); iii) Good or fair trophectoderm (TE) (grade A and B). Blastocysts, which did not reach our morphological requirements, were discarded. The scoring of fragmentation rate and the number of blastomeres at 45 h PI was not considered.

Culture slides were screened for blastocysts from well number 1 to well number 12. The observed good morphology blastocysts were vitrified in the same order.

2.5. Vitrified blastocyst transfer (VBT)

Good morphology blastocysts were placed individually in droplets of Equilibration Solution (IrvineScientific, USA) for 10 min and then placed in a droplet of Vitrification Solution (IrvineScientific) for 60 s [20]. Blastocysts were then loaded on Rapid-i™ Vitrification System (Vitrolife) and stored in LN_2. Warming was obtained placing the blastocyst in 500 μL of Thawing Solution (IrvineScientific) for 1 min, transferred in two 60 μL droplets of Dilution Solution (IrvineScientific) for 2 min each and then washed three times in 60 μL droplets of Washing Solution (IrvineScientific).

Blastocysts were selected for transfer by order of vitrification. Since vitrification order was based on the random placement of zygotes in the culture slide, e.g. the blastocyst in well number 1 was vitrified and transfer as first. Our blastocyst transfer model could thus be considered unbiased by morphology selection. A cycle of warming and subsequent transfer of a single vitrified blastocyst was defined as a vitrified blastocyst transfer (VBT). One blastocyst per VBT cycle was warmed and incubated for 4 h. Only blastocysts showing expansion activity were then transferred.

In total we assessed 238 vitrified-warmed and transferred blastocysts, which resulted in 59 live births. This group was defined "cumulated VBT group". Moreover, we assessed a subgroup, which included only the first blastocyst that each patient received. This subgroup was defined as "first VBT subgroup" and included 152 single blastocysts, which resulted in 49 live births.

2.6. Time-lapse assessment

The time-lapse recordings of all vitrified-warmed and transferred blastocysts were reassessed by two operators not involved in embryo selection for fresh transfer. In case of disagreement between the operators, the blastocyst was reassessed and a joint assessment was agreed. Embryos were observed from zygote to compaction. All cell divisions were recognized and assessed.

Assessment of cell division was performed as previously described [21]: Regular cell divisions were defined as the synchronized occurrence of cytokinesis and mitosis producing two daughter cells. The beginning of a cell division was recognized by the beginning of the mitosis, i.e., the disassembling of the nucleus, and the completion by the end of the mitosis, i.e., the reassembling of the nuclei.

Cell divisions producing more than 2 nucleated cells were defined as multi-cell divisions, while cell divisions producing less than 2 nucleated cells were defined as failed cell divisions. Failed cell divisions included five patterns, based on the cytokinesis the number of the nuclei reassembled: 1) Sibling blastomeres recombining after completion of cytokinesis, and reassembling of two nuclei; 2) Absence of cytokinesis, and reassembling of two nuclei; 3) Cytokinesis producing large amount of fragments, i.e. ≥30% of the parent blastomere volume, and a single blastomere with two nuclei; 4) Cytokinesis producing large amount of fragments, i.e. ≥30% of the parent blastomere volume, and a single blastomere with one nucleus.

Multi-cell divisions and failed cell division were considered as ACDs.