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ORIGINAL ARTICLE

Relationship between pregnancy, embryo development, and sperm deoxyribonucleic acid fragmentation dynamics

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Abstract The way the dynamics of DNA fragmentation affects the growth of embryos in real time, and effectiveness of infertility treatment using the ICSI procedure were determined in 148 couples treated with the ICSI technique. The percentage of sperm with fragmented DNA (known as the DNA fragmentation index [DFI]) in semen samples was determined at 3, 6 and 12 h. Embryo culture was assessed continuously during 12 h of observation monitoring.

Statistically significant difference was found in DFI at 12 h and outcome of treatment. For the remaining time intervals, no statistically significant differences were noted. An analysis of relationship between the DFI dynamics over time at individual measurements and achievement of pregnancy, confirmed a statistically significant relationship between the rate measured at 6–12 h of observations of DFI changes (DFI 12 h%/h), and achieving pregnancy. Correlation was observed between DFI (during 0, 3, 6 and 12 h), the growth rate in DFI, and time of embryo development. A statistically significant relationship was found between the rate from the start to the end of observations of the DFI, and outcome of treatment.

Intensity level regarding fragmentation of sperm DNA and its growth rate affected the time of embryo development in the ICSI procedure. The most significant prognostic factor for achieving pregnancy was intensification of sperm DNA fragmentation after 12 h.

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1. Introduction

Obtaining pregnancy in the treatment procedure in vitro depends mainly on the quality of the embryo and oocyte. The reproductive potential of the sperm is conditioned not only by its morphologically normal structure and motility, but also sperm chromatin lesions to which belongs, among

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others, DNA fragmentation (Erenpreiss et al., 2006). It is known from the literature that the spermatozoon, even with considerably intensified DNA fragmentation, is able to fertilize an oocyte (Erenpreiss et al., 2006). The oocyte also possesses the ability to repair abnormal DNA; however, this depends on the type of damage, as well as the quality of the oocyte.

Sperm DNA fragmentation is not a static phenomenon, it is intensified in time and to various degrees, varies between individuals, and according to the external factors exerting an effect on sperm, related primarily with oxidative stress. The dynamics of DNA fragmentation may depend on the method of sperm preparation prior to its use for assisted reproductive technology (ART). The ultimate degree of DNA fragmentation also depends on the time elapsed from the collection of sperm until the use of a spermatozoon for ART procedures (Gosálvez et al., 2011b; Perrault et al., 2003; Evenson and Wixon, 2005; García-Contreras et al., 2011; Tremellen, 2008).

Therefore, it may be presumed that the quality of genetic material in sperm, which depends, among other things, on the level of intensity of nuclear DNA fragmentation, will exert a direct effect on the development of the embryo obtained as a result of the intracytoplasmic sperm injection (ICSI) procedure. For a long time, the quality of an embryo has been evaluated based on only morphological criteria, which seem to be insufficient to prognosticate the possibilities of achieving pregnancy after its transfer (Guerif et al., 2010; Arav et al., 2008). An alternative to morphological evaluation of an embryo is the observation of its development in real time due to the technology consisting in placing a camera inside the incubator (Arav et al., 2008; Cruz et al., 2011; Pribenszky et al., 2010; Wale and Gardner, 2010).

The objective of the presented study is to determine in what way the dynamics of DNA fragmentation affects the growth of embryos in real time, and the effectiveness of infertility treatment using the ICSI procedure.

2. Materials and methods

The study was conducted in 2012 and 2013, in the Non-Public Health Care Unit 'Ovum Reproduction and Andrology' in Lublin, and covered 148 couples treated due to infertility by the ICSI technique. The sperm DNA fragmentation index (DFI) was examined on the day of microinjection, as well as the dynamics of fragmentation (DFI-h), and retrospectively, the outcomes of treatment of the couples were evaluated: timing of embryo development and achievement of clinical pregnancy.

Female inclusion criteria were: age 25–35 years, FSH (follicle-stimulating hormone) <10 IU/ml and AMH (Anti-Mülerian Hormone) >1.5 ng/mL. Female exclusion criteria were: BMI (body mass index) <17 and >30, metabolic diseases. Male inclusion criteria were: age 25–35 years, treatment for infertility >1 year (couples had 4–6 prior intrauterine inseminations performed, males used dietary supplements in order to improve sperm parameters). Duration of the period preceding the treatment procedure was 1–4 years. Exclusion criteria for males were: severe asthenoteratozoospermia, symptoms of systemic diseases, inflammatory state of the reproductive organ, smoking BMI (Body Mass Index) <17 or >30, pre- and post-natal developmental disorders in reproductive organs, varices of the spermatic cord, taking medicines that may affect the quality and density of sperm up to 3 months prior to the study.

All patients signed a written consent to participate in the study. The study was approved by the Bioethics Commission.

In all patients, treatment with the ICSI procedure was applied using fresh oocytes and spermatozoa (sperm density $\geqslant 1$ million/ml). Sperm was obtained by masturbation three hours before microinjection and was examined directly after liquidation according to WHO criteria. Before the examination, males abstained from sex and alcohol for 3–4 days. In order to determine the percentage of sperm cells with fragmented DNA, the sperm chromatin dispersion test (SCD) was used, according to the instruction supplied with the kit (Dyn-Halosperm® kit, Halotech DNA SL, Madrid, Spain) (Fernández et al., 2005).The examination resulted in obtaining the sperm DFI – the percentage of sperm with DNA fragmentation. DFI was determined in sperm samples directly after liquidation (DFI 0 h), and subsequently after 3 h (DFI 3 h), 6 h (DFI 6 h), and 12 h (DFI 12 h) incubation in an automated incubator with 5% CO_2 at a temperature of 37 °C. On this basis, the dynamics over time between individual measurements was calculated (in percentages per hour).

DFI (3 h%/h) – is the % DFI per hour during the first 3 h, DFI (6 h%/h) – is% DFI per hour during the subsequent 3 h, and so on at individual intervals. Also, the rate of DFI increase was determined over the entire examination time from 0 to 12 h – DFI (%/h).

Ovarian stimulation was performed by the administration of gonadotropin-releasing hormone (GnRh) analog (Diphereline: Ipsen Pharma), followed by recombinant FSH (Gonal-F: Merck-Serono, Puregon: Organon) from cycle day 3 in a short protocol. The aspiration of oocytes was performed 36 h after the administration of recombinant HCG (r-hCG) (Ovitrelle: Merc-Serono).

After aspiration oocytes were placed in Fertilization medium (COOK, Sydney IVF, Australia) under mineral oil and after retrieval up to six oocytes (in accordance with Polish law) were subjected to ICSI procedure.

Oocytes were denuded from the granular layer and ICSI was performed 3 h after follicular puncture; fertilized cells were cultured in 25 µl drops of Cleavage medium (COOK, Sydney IVF, Australia) under mineral oil until day 2 (2–5 cell stage) in an automated incubator with 5% CO_2 at 37 °C fitted with time-lapse image acquisition (Time-lapse, Primo Vision EVO Microscope, Cryo-Innovation, Hungary). Fifty hours after ICSI, the culture media were changed to Blastocyst medium (COOK, Sydney IVF, Australia).

The growth of embryos was evaluated by monitoring at 10-min intervals using a camera placed inside the incubator. During the observation the embryos were not taken out of the incubator. Between image acquisitions the monitoring system was turned off to avoid exposure to electromagnetic radiation.

The 0 time was defined as the time of ICSI; the tF was defined as the time of the first frame in which the pronuclei were observed, while the tC as the time of the frame with the last observation. The stage of unicellular embryo after syngamy was defined as t1, and the subsequent stages were marked as t2, t3, t4, t5, t6, t7, t8. The time to the onset of formation of morula was defined as tM, while tB was the time in which a crescent-shaped area began to emerge from the