Article

Microtubule turnover in ooplasm biopsy reflects ageing phenomena in the parent oocyte

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Abstract

Oviductal oocytes retrieved from superovulated B6D2F1 mice at 13.5, 16 and 19 h after human chorionic gonadotrophin (HCG) (groups A, B and C respectively, n = 382) were micromanipulated to obtain 12–20 µm sized ooplasm biopsy fragments. Experiments were divided into three sets. Ooplasmic microtubule dynamics were studied in ooplasm biopsy specimens and parent oocytes (set 1) and ooplasm biopsy specimens (set 2), whilst zona pellucida dissolution time, cortical granule loss and spindle/chromatin morphology using confocal microscopy were also studied in parent oocytes (set 2). Oocytes withstood oocyte biopsy with a high survival rate (98.2%) and the biopsied oocytes underwent successful fertilization and development (set 3). An absolute one-to-one correlation was seen between the oocyte biopsy specimens and the parent oocytes in terms of ooplasmic microtubule dynamics (set 1), and increased ooplasmic microtubule dynamics in oocyte biopsy specimens paralleled ageing phenomena in the parent oocytes (set 2). Zona pellucida dissolution time was significantly lower in parent oocytes from group A versus groups B (P = 0.032), and C (P < 0.001). (Groups A, B, C include minimal, moderate, increased ooplasmic microtubule dynamics in oocyte biopsy specimens respectively.) Oocyte cortical granule loss and spindle/chromatin abnormalities were mainly seen in group C (P < 0.001). Oocyte biopsy can thus be applied to judge age-related changes in the parent oocytes.

Keywords: *cortical granules, ICSI, microtubule dynamics, oocyte, ooplasm biopsy, post-ovulatory ageing, spindle*

Introduction

Achievement of success in obtaining a viable pregnancy while avoiding multiple order births is a major challenge in assisted reproduction treatment programmes today. Both of these goals could be achieved by transferring to the uterus a single embryo with known high implantation potential (Ozturk *et al*., 2001). However, there are limitations to optimal embryo selection, since there are no current methods that guarantee improved developmental potential.

The contemporary approach to embryo selection is based on examining the morphology and rate of in-vitro development of the oocyte/embryo (Saith *et al*., 1998). Nevertheless, this method does not definitively predict the success of implantation or continual development. Hence, more than one embryo is generally transferred to the subjects undergoing assisted reproduction. As a result, there has been a significant increase in multiple gestations in addition to higher pregnancy rates in the SART registry (Reynolds *et al*., 1997).

Research in the area has continued over the years, and the concept of 'oocyte and embryo quality' has emerged. The term 'quality' refers not only to the structural integrity, but also to the normality of chromosomes, metabolism, and developmental potential. Although direct and indirect

techniques such as embryo blastomere or polar body biopsy are available to assess chromosomal numbers, they have certain limitations, and their use has generally been confined either to women with advanced age or to couples with known genetic disorders (Verlinsky *et al.*, 1998; Wilton *et al.*, 2002). There are no established clinically useful methods to assess the oocyte/embryo metabolism and developmental potential so far.

Oocyte pre-/post-ovulatory ageing is known to cause abnormal fertilization as well as developmental compromise (Chang *et al.*, 1958; Gray *et al.*, 1984; Wilcox *et al.*, 1998; Goud *et al.*, 1999). It is also possible that within a cohort of oocytes obtained at oocyte retrieval, there may be certain oocytes that have impaired fertilization and developmental potential, secondary to being aged or having a higher likelihood to undergo ageing. Furthermore, the cytopathology and to some extent, the pathophysiology of oocyte ageing are relatively well understood (Igarashi *et al.*, 1997; Xu *et al.*, 1997; Takahashi *et al.*, 2003). This study therefore used oocyte ageing as a model abnormality, and applied a newly developed technique to obtain a viable ooplasm sample to assess oocyte ageing.

Microtubules are components of cytoskeleton involved in various functions, including organelle trafficking and chromosome separation at cell division. Microtubules are made up of tubulin monomers that undergo dynamic changes of polymerization and depolymerization, and finally reach a steady state of microtubule turnover. This steady state is dependent on the cellular metabolism. The critical concentration for tubulin polymerization is affected by various factors, including the cell cycle stage (Alberts *et al.*, 1994). Remarkably, ooplasmic microtubule turnover increases with post-ovulatory ageing (Pickering *et al.*, 1988; Zernicka-Goetz *et al.*, 1993; George *et al.*, 1996). This increase can be made obvious by exposing oocytes to taxol, which enhances microtubules in the ooplasm of post-ovulatory old, but not young oocytes (Goud *et al.*, 2004).

Ooplasm biopsy could be representative of the parent oocyte, and increased microtubule dynamics in the ooplasm biopsy may reflect ageing related phenomena of the parent oocyte. This study therefore examined the response of ooplasm biopsies and parent oocytes to taxol and used 'taxol-enhanced microtubule dynamics' in the ooplasm biopsy (Goud *et al.*, 2004) as a marker to predict ageing related changes in the parent oocyte.

Materials and methods

Study design

Approval for the current study was obtained from Wayne State University's Animal Investigation committee. Design of the study involved obtaining MII stage oocytes from superovulated mice at 13.5, 16 and 19 h after human chorionic gonadotrophin (HCG). In experiment set 1, normal appearing MII stage oocytes obtained from B6D2F1 mice were divided into treatment and control groups. The oocytes in the treatment group were subjected to the micromanipulation procedure of ooplasm biopsy. The biopsy fragment, and the parent and control oocytes were all treated with taxol. Microtubules were examined after α-tubulin fluorescence immunocytochemistry and confocal microscopy. In experiment set 2, oocytes were obtained and divided in the same way as set 1, and the oocytes in the treatment subgroup were subjected to ooplasm biopsy. Only the ooplasm biopsy fragments were treated with taxol prior to tubulin immunocytochemistry, whereas the parent oocytes were processed for cortical granule, tubulin, and chromatin staining after assessment of zona pellucida (ZP) dissolution time. The operator was blinded to oocyte age prior to ooplasm biopsy and assessment of ooplasmic microtubule dynamics. In experiment set 3, oocytes were subjected to ooplasm biopsy followed by intracytoplasmic sperm injection (ICSI), and followed through fertilization and development in culture.

Superovulation and oocyte retrieval

Four- to 6-week-old B6D2F1 mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA), and were adjusted to a 14-h light–-10-h dark cycle for at least 1 week prior to superovulation with 7.5 IU each of pregnant mare's serum gonadotrophin (PMSG) and HCG (Sigma, Saint Louis, MO, USA), administered IP 48–52 h apart. Mice were killed 13–19 h after HCG injection and oocytes were retrieved from their oviductal ampullae. The cumuli were treated with 0.1% hyaluronidase (w/v) in M2 medium (Sigma) for 2–3 min at 37°C to release oocytes, which were subsequently denuded to remove all cumulus–corona cells with a narrow bore pulled glass Pasteur pipette. Oocytes were thoroughly rinsed in M2 medium, inspected to rule out abnormal morphology and were kept ready in M16 medium (Sigma) pre-equilibrated with 5% CO_2 in air at 37°C for subsequent procedure with or without micromanipulation.

Micromanipulation set-up and procedure

The set-up used for the micromanipulation procedure was similar to that used for embryo-biopsy and preimplantation diagnosis (Dozortsev and McGinnis, 2001), except for some differences in the dimensions of the micro-tools. The external and internal diameters of the holding pipettes were ~75–80 μm and 10–12 μm respectively and those for the embryo biopsy (EB) micropipettes were 20–25 μm and 12–19 μm respectively. Micromanipulations were performed on a warm stage (37°C) of a Nikon Diaphot microscope equipped with coarse hydraulic micromanipulators (Narishige, Tokyo, Japan).

The micromanipulation procedure for ooplasm biopsy was performed as described below (**Figure 1A–L**). The oocytes were steadily held with the holding pipette and a partial zona dissection was performed using a sharp tipped partial zona dissection micropipette at a pole away from the polar body and the spindle area. Care was taken to maintain the same orientation in each oocyte. The oocyte position was then readjusted using the holding micropipette, and an EB micropipette was inserted into the perivitelline space gently abutting against the oolemma. The ooplasm was carefully aspirated without puncturing the oolemma. This was followed by a gentle withdrawal of the EB pipette, thereby stretching the ooplasm to a point where the ooplasm fragment broke from the parent oocyte. The oolemma enveloped ooplasm fragment that was then allowed to