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# Cytoplasm replacement following germinal vesicle transfer restores meiotic maturation and spindle assembly in meiotically arrested oocytes

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Dr Zhang completed his medical degree in at the Zhejiang University School of Medicine, and subsequently received his Master's Degree at Birmingham University in the UK. In 1991, Dr Zhang earned his PhD. in In-Vitro Fertilization (IVF), and, after studying and researching the biology of mammalian reproduction and human embryology for nearly ten years, became the first Fellow in the Division of Reproductive Endocrinology and Infertility of New York University's School of Medicine in 2001. Dr Zhang continues his research in non-embryonic stem cell research, long-term cryopreservation of oocytes, and oocyte reconstruction by nuclear transfer.

**Abstract** Both the cytoplasmic and nuclear compartments are essential for the acquisition of meiotic competence. This study assessed the role of the cytoplasm in meiosis resumption in meiotically arrested oocytes at the germinal vesicle (GV) stage. Mouse oocytes at GV stage were meiotically arrested with 3-isobutyl-1-methylxanthine (IBMX). GV transfer was performed between IBMX-treated and non-treated (control) mouse oocytes, and between control mouse and human GV oocytes. Extrusion of first polar body (PB) was examined as an indication of nuclear maturation. Meiotic spindle assembly and chromosome alignment were examined by immunostaining. Results indicated that oocytes arrested with IBMX for 24 and 48 h exhibited reduced ability for meiotic maturation and for extruding the first PB when compared with controls ($P < 0.01$). IBMX-treated oocytes reconstituted with cytoplasm, but not GV, of control oocytes restored the assembly of meiotic spindle and meiotic maturation. Mouse oocytes reconstituted with GV of human oocytes underwent meiosis similar to that observed in mice, but not humans. Additionally, human oocytes reconstituted by mouse GV underwent meiosis similar to that observed in humans, but not mice. These findings suggest that cytoplasm replacement by GV transfer could represent a potential therapeutic option for women who do not produce mature oocytes during IVF.


**KEYWORDS:** cytoplasm, germinal vesicle, meiotic maturation, meiotic spindle, nucleus, oocyte

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## Introduction

The mammalian ovary contains a large supply of inactive germ cells that reside in primordial follicles and two categories of oocyte. The first category constitutes oocytes that are unable to respond to maturation signals *in vivo* or *in vitro* and remain arrested in the diplotene stage (Fulka et al., 1998). The second category makes up a fully grown group of oocytes that respond to gonadotrophins and mature to metaphase II in pre-ovulatory follicles and in culture (Fulka et al., 1998). Oocyte maturation is a complex process involving both the progression of the meiotic cycle and the reprogramming of cytoplasmic events (Fulka et al., 1998; Karnikova et al., 1998; Liu et al., 1999; Moor et al., 1998). Some of the structural changes in the cytoplasm include an increase in the number of mitochondria, structural modification to the Golgi apparatus and accumulation of ribosomes (Fulka et al., 1998; Heacox and Schroeder, 1981). The end-point of oocyte maturation is the release of a mature metaphase II (MII) oocyte that is competent to support normal embryonic development. Both nuclear and cytoplasmic deficiencies have been shown to be responsible for poor oocyte quality by contributing to meiotic defects and subsequent impaired embryo development (Fulka et al., 2001; Liu et al., 2000, 2003; Liu and Keefe, 2004; Moor et al., 1998).

Germinal vesicle (GV) transfer techniques have represented useful tools for studying the interaction between the nucleus and the cytoplasm in the oocyte maturation process in mammals (Chiang et al., 2012; Cohen et al., 1997; Dekel and Beers, 1978; Fulka et al., 1998, 2001; Heacox and Schroeder, 1981; Levron et al., 1996; Li et al., 2001; Liu et al., 1999, 2000; Zhang et al., 1999). Furthermore, GV transfer between different mammalian species has demonstrated that cytoplasmic factors regulating the progression of the first and the second meioses are not species-specific in mammalian oocytes and that these factors are located in the meiotic apparatus and/or its surrounding cytoplasm at the MII stage (Li et al., 2001). It has previously been reported in humans (Zhang et al., 1999) and mice (Liu et al., 2000, 2003) that normal meiosis can occur after the transfer of GV into an enucleated host oocyte. It has also been shown in mice that oocytes reconstructed by GV transfer into a cytoplasm of the same developmental stage mature normally *in vitro* through MII (Liu et al., 1999). Studies to date have evaluated the in-vitro maturation capability of reconstructed oocytes by GV transfer (Heacox and Schroeder, 1981; Li et al., 2001; Zhang et al., 1999) but did not address data on meiotic maturation such as polar body (PB) extrusion, meiotic spindle assembly or chromatid separation.

Abnormal oocyte spindle morphology is associated with human female infertility, and advanced maternal age has been attributed to spindle abnormalities such as abnormal chromosome alignment and a microtubule matrix that compromises the meiotic spindle (Battaglia et al., 1996). The regulatory mechanisms responsible for assembly of the meiotic spindle in the cytoplasm are significantly altered in older women, leading to high prevalence of aneuploidy (Battaglia et al., 1996). Whether the GV transfer technique could represent a therapeutic option for meiotic spindle abnormalities remains to be determined. It is well established that pharmacological activation of cAMP-dependent protein kinases by 3-isobutyl-1-methylxanthine (IBMX) suppresses meiotic resumption (Dekel and Beers, 1978). The present authors (Liu et al., 2003) and others (Eppig and Schroeder, 1989) have demonstrated that mouse oocytes treated with IBMX at the GV stage *in vitro* have arrested during their meiotic maturation, and when released from IBMX were able to produce normal embryonic development. The purpose of this study was to explore the role of the cytoplasm on meiotic spindle assembly in oocytes arrested by IBMX *in vitro* and to assess whether cytoplasm replacement via GV transfer restores meiotic maturation, in particular spindle assembly in the IBMX-arrested oocytes. We postulated that a healthy cytoplasm is required for GV breakdown and meiosis resumption.

## Materials and methods

### Mouse oocytes

The CB6F1 mice used in this experiment were purchased from Charles River Laboratory (Boston, MA). Mice were subjected to a 14L:10D cycle for at least 1 week before use. Animals were cared for according to the procedure approved by University Animal Welfare Committee (UAWC). GV-stage oocytes were collected from 6- to 8-week-old CB6F1 mice through puncturing the ovarian follicles 48 h following priming with 5 IU pregnant mare serum gonadotrophin (PMSG, Sigma, St Louis, MO). GV oocytes, stripped off cumulus granulosa cells with a diameter around 80 μm, were used in this experiment. GV oocytes were incubated in human tubal fluid (HTF) medium supplemented with 10% fetal calf serum (FCS) (HyClone, ThermoFisher Sceintific, Waltham, MA) and 50 μg/ml of IBMX (Sigma, St Louis, MO) constituting the treatment group, thus preventing GV from breakdown by increasing cytosolic cAMP following inhibition of phosphodiesterase. Mouse oocytes were arrested at GV stage *in vitro* following culture in IBMX for 6, 24 and 48 h, respectively. The arrested oocytes were then released from IBMX by changing the medium to IBMX-free medium. The dose of IBMX used was chosen from previously published data (Liu et al., 2003; Van Cauwenberge and Alexandre, 2000). These treatments were used for various periods of time and then GV were released from IBMX and cultured in IBMX-free HTF medium (F-HTF) for 15 h for meiosis resumption. Oocytes without IBMX treatment constituted the control group.

### Human GV oocyte

GV-stage oocytes were obtained from patients (25–42 years old) who were undergoing IVF with intracytoplasmic sperm injection (ICSI) after ovarian stimulation with gonadotrophins. All patients were treated with human chorionic gonadotrophin (HCG; 5000 or 10,000 IU) 36 h before transvaginal oocyte retrieval. Institutional Review Board (IRB) approval was obtained on July 23 1998 from New York University School of Medicine (NYUMC-IBRA Protocol H 6902). Following an IRB approval, participants consented and GV oocytes that were unsuitable for ICSI and routinely discarded were used in this study.

### GV transfer and electromembrane fusion

Oocytes were exposed to modified HTF medium (mHTF) with 10% FCS supplemented with 7.5 μg/ml cytochalasin B (CB;