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SHORT COMMUNICATION

Human oocytes containing large cytoplasmic vacuoles can result in pregnancy and viable offspring

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Abstract The effect of oocyte dysmorphism on further embryo development is controversial. It is generally accepted that serious oocyte abnormalities can have a negative effect on further fertilization and development. A couple reported to the clinic following 2 years of infertility and underwent five IVF/intracytoplasmic sperm injection treatments due to severe male factor infertility. A total of 42 oocytes were collected. The majority of the oocytes showed at least one large, fluid-filled and centrally located cytoplasmic vacuole and unusually thin zona pellucida. Only seven oocytes showed normal fertilization. The first four IVF treatments did not result in pregnancy. In the fifth IVF treatment, three poor-quality vacuolized embryos were transferred. A singleton pregnancy was detected. A baby girl was born at term who required surgery because of a double left kidney and ureter. This case report demonstrates that serious oocyte abnormalities can be a recurrent phenomenon in the same patient. However, the presence of a large vacuole does not completely block the fertilization process and this abnormal cohort of oocytes can still result in normal embryo development and a viable offspring. Rigorous prenatal care and follow-up should be carried out following the transfer of embryos developed from dysmorphic oocytes.
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Introduction

Oocyte morphology strongly influences the viability and developmental competence of human preimplantation embryos in assisted reproductive treatments. Oocyte dysmorphisms can be divided into cytoplasmic anomalies, such as increased granularity, presence of vacuoles, refractile bodies, and extracytoplasmic anomalies, such as irregular shape or thickness of zona pellucida, enlarged perivitelline space or abnormal morphology of the first polar body (Balaban and Urman, 2006; Ebner et al., 2003). It has been

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reported that cytoplasmic inclusions resulted in a lower fertilization rate and unfavourable embryo quality (Otsuki et al., 2007; Xia, 1997); however, contradictory data have also been published (Balaban et al., 1998; De Sutter et al., 1996). Multiple oocyte anomalies (vacuoles and inclusions) were also related to decreased fertilization and pregnancy rate (Loutradis et al., 1999). This negative impact on embryo viability may be explained by a higher rate of aneuploidy in dysmorphic oocytes (Kahraman et al., 2000; Plachot, 2001).

One of the most common oocyte dysmorphisms is cytoplasmic vacuolization. Vacuoles are membrane-bound cytoplasmic inclusions filled with fluid that is virtually identical with perivitelline fluid. They vary in size as well as in number and can be observed in 5–12% of oocytes (Ebner et al., 2006). Vacoules arise either spontaneously or by fusion of preexisting vesicles derived from Golgi apparatus or smooth endoplasmatic reticulum (El Shafie et al., 2000; Van Blerkom, 1990). It has been shown that vacuolated oocytes have significantly reduced fertilization rates (Rienzi et al., 2008) and developmental ability. Moreover, a vacuole >14 μm in diameter can completely block fertilization (Ebner et al., 2005). Determining the effect of a single oocyte dysmorphism is quite difficult because many oocytes show more than one morphological abnormality (De Sutter et al., 1996). It has also been reported that serious oocyte dysmorphisms, such as clusters and vacuoles in the cytoplasm, can be a recurrent phenomenon in consecutive IVF treatments for the same patient (Akarsu et al., 2009; Wallbutton and Kasraie, 2010). In these cases, it is often difficult to counsel patients as to how to proceed.

This case report describes the successful treatment of an infertile couple where a recurrent serious vacuolization was observed over five consecutive IVF/intracytoplasmic sperm injection (ICSI) treatments.

Case report

A couple visited our clinic after 2 years of infertility. The female partner was 36 years old and had secondary infertility. A pregnancy from a previous partner was terminated artificially. The anatomy of the uterus was normal but the left tube was blocked. Baseline hormonal assessment showed normal values. The male partner, aged 46 years, presented with severe oligoasthenoteratozoospermia.

The couple underwent five consecutive IVF/ICSI cycles within 2.5 years. Standard long-protocol stimulation was performed using gonadotrophin-releasing hormone agonist triptorelin (Decapeptyl; Ferring, Kiel, Germany) for down-regulation followed by urinary gonadotrophins or recombinant FSH ovarian stimulation in all five cycles (Fostimon, IBSA, Lugano, Switzerland; Gonal-F, EMD Serono, Geneva, Switzerland; Menogon, Ferring; Menopur, IBSA). The type of gonadotrophin used for ovarian stimulation was always the same in one stimulation cycle. Oocyte collection, ICSI treatment, embryo culture and embryo transfer were performed using standard procedures.

During the five cycles, 42 oocytes (range 5–13, average 8.4/cycle) were collected. Maturity and morphology of the oocytes was assessed and recorded before sperm injection. Pictures of the oocytes were also recorded and measurements were taken using Octax Eyewere Imaging software (MTG, Bruckberg, Germany). Summarizing the characteristics of all oocytes collected in the five cycles, 76.2% of the oocytes (range 5–9, average 6.4/cycle) were mature (metaphase II) at the time of sperm injection, 9.5% were immature and 14.3% were degenerated. The rate of oocyte immaturity was similar but the rate of degenerated oocytes was much higher than normal rates observed at the study centre over the same period (14.3% versus 2%; $P < 0.001$). Most of the mature oocytes (81.3%) contained at least one large, centrally located, fluid-filled cytoplasmic vacuole (**Figure 1**A–C), 29.2–61.0 μm in diameter (52.2 μm average). Also, 75% of the oocytes had a fragmented or enlarged first polar body. The zona pellucida was also extraordinarily thin in most of the oocytes, the average thickness was 9.7 μm which is significantly different from the average zona thickness of normal oocytes (18.2 μm; $P < 0.001$) collected in the department.

All 32 mature oocytes were injected. Seven zygotes showed normal fertilization (22%) and one zygote showed one pronuclear fertilization and 24 oocytes (75%) did not show any sign of fertilization. Most of the embryos had poor morphology before the embryo transfer, but there was at least one embryo available for transfer in each cycle (1, 1, 2, 1 and 3 embryos, respectively). The first four IVF cycles did not result in a pregnancy. In the fifth IVF cycle two oocytes were fertilized normally (**Figure 1**D and E) and one oocyte showed one pronuclear fertilization (Figure 1F). These zygotes reached the 2–4-cell stage on day 2 and all of them still contained large vacuoles (**Figure 1**G–I). These embryos were transferred according to the centre's transfer policy and resulted in a singleton pregnancy with fetal heart activity. During prenatal care, the fetus was found to have a double left kidney and ureter but was otherwise healthy. No prenatal nor postnatal karyotyping was performed on the child according to the parents' decision. A baby girl of weight 3200 g and height 55 cm was delivered by Cesarean section in week 39 of pregnancy. Her kidney disorder was treated by surgery at 1 year of age without requiring any other interventions or medications and up to now she is healthy.

Discussion

As far as is known this is the first case in the literature reporting a pregnancy and viable offspring following IVF treatments of a woman who had large vacuoles in most of the oocytes collected in consecutive ICSI cycles. The most remarkable phenomenon in these oocytes was the large, fluid-filled and centrally located vacuole. This serious dysmorphism was associated with irregularly thin zona pellucida and fragmented or enlarged first polar bodies in most of the oocytes. The first polar body can fragment or degenerate during post-ovulatory aging of oocytes (Ebner et al., 1999; Eichenlaub-Ritter et al., 1995). Thus, a fragmented or enlarged first polar body is related to decreased fertilization ability and viability (Ebner et al., 1999; Fancsovits et al., 2006; Rienzi et al., 2008).

Oogenesis and oocyte maturation may be affected by ovarian stimulation. There are, however, very few and conflicting studies examining the effects of different gonado-

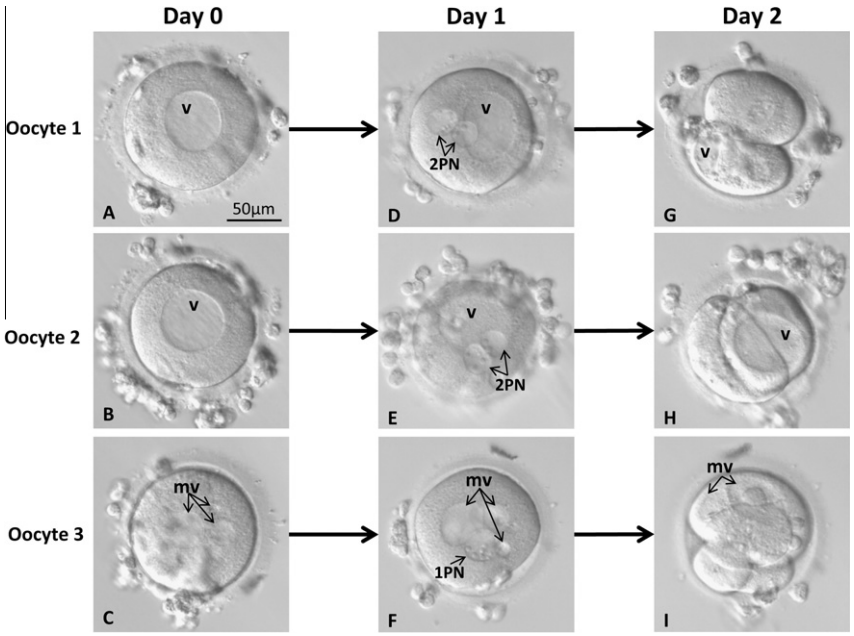

Figure 1 Fertilization and cleavage of oocytes showing one (A and B) or multiple (C) large vacuoles and extremely thin zona pellucida. Oocytes 1 and 2 had normal (2PN) fertilization (D and E) while oocyte 3 resulted in 1PN fertilization (F). Embryos with two (G and H) or four (I) blastomeres were transferred and resulted in singleton pregnancies. mv = multiple vacuoles; v = vacuole; 1PN = one pronucleus; 2PN = two pronuclei.

trophin preparation on oocyte morphology and quality. Imthurn et al. (1996) reported that cytoplasmic appearance of oocytes is influenced by the gonadotrophin preparation used for ovarian stimulation. However, other studies did not confirm this finding when analysing the effect of hormonal stimulation on the oocyte morphology (Murber et al., 2011; Ng et al., 2001; Rashidi et al., 2005).

The multiple anomalies and the high number of degenerated oocytes indicates that these oocytes endured serious intrinsic adverse effects during oogenesis and maturation. The present report confirms previous findings (Akarsu et al., 2009; Wallbutton and Kasraie, 2010) and clearly shows that oocyte anomalies can be a recurrent phenomenon in the same patient.

Several authors have demonstrated that such a dysmorphic oocyte has zero or minimal chance of fertilization and normal development (Ebner et al., 2005; Wallbutton and Kasraie, 2010) which was confirmed by the extremely low (22%) fertilization rate in the present case. Single or multiple large vacuoles may displace the meiotic spindle from its polar position or disturb the cytoskeleton resulting in fertilization failure (Ebner et al., 2005; Van Blerkom, 1990).

In spite of this, some of the oocytes fertilized and underwent further cleavage cycles in the present case. However, vacuoles were observed in cells throughout development. The effect of serious oocyte anomalies on further embryo development and implantation is rather controversial in the literature. It has been reported that oocytes with granular cytoplasm or irregular thickness and shape of zona pellucida resulted in normal fertilization, implantation and a viable offspring (Esfandiari et al., 2005; Paz et al., 2004). Esfandiari et al. (2006) published a case where oocytes with serious multiple abnormalities (granular and dark cytoplasm, dark and thick zona pellucida) were able to fertilize, cleave and develop to a viable offspring. Oocytes containing large smooth endoplasmic reticulum aggregations also resulted in clinical pregnancies, but chromosomal abnormality and multiple fetal anomalies were described in these cases (Akarsu et al., 2009). The present report clearly demonstrates that oocytes showing large, centrally located, fluid-filled vacuoles, abnormal first polar bodies and zona pellucida can be normally fertilized, resulting in viable offspring. Rigorous prenatal care and follow-up should be carried out following the transfer of embryos developed from dysmorphic oocytes.

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