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CASE REPORT

A Case Study on Vacuolated Oocytes Intracytoplasmic Sperm Injection and its Outcome

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ABSTRACT

This is a case study showing effects of oocyte morphological abnormalities in the form of homogenous vacuoles on intracytoplasmic sperm injection (ICSI) outcomes and its implications. Characteristic vacuoles of different size were identified in all oocytes obtained after egg collection in this patient. Eighteen oocytes were retrieved, 15 were metaphase-II and three were metaphase-I. All the oocytes were injected with sperm; out of 18 oocytes, 12 oocytes had embryo quality of grade A and three were grade B. Fifteen embryos exhibited maturation on 24 to 36 hours of incubation and showed two to four-celled stages, and after 48 to 60 hours of incubation, the embryos showed six to eight-celled stage. Grade A quality of three embryos was transferred nearly in an ongoing singleton pregnancy was confirmed.

Keywords: Oocyte, Short protocol, Vacuole.

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INTRODUCTION

The development of oocyte competence dependent on a complex several cascade of molecular pathways will happen during follicular development. In the course of development of these competencies, cytoplasmic changes happen that consists of mRNA transcription, protein translation, posttranslational modification of proteins, and ultrastructural changes.[1,2] Cytoplasmic maturation is referred as successful end of cellular events and is independent of nuclear maturation.[3]

An oocyte develops and matures, it possesses the capacity to complete meiosis,[4] successfully go through the fertilization process, and initiate and sustain embryonic development.[1-3] Cytoplasmic maturation of an oocyte that has not completed is of bad quality and thus inefficient for normal developmental processes. However, the mechanisms that weaken oocyte developmental competence are still not understood.[3] Gonadotropins are used in assisted reproductive treatments for oocyte maturation and are induced via the administration of exogenous human chorionic gonadotropin (hCG) for follicle stimulation.

Intracytoplasmic features (increased cytoplasmic granularity and presence of cytoplasmic inclusions) or extracytoplasmic features [large periviteline space (PVS), PVS granularity, and fragmented or IPB (irregular polar bodies)] based on these morphological malformations of oocytes are mostly classified.[5] It is observed that oocyte vacuolization is common in clinical. It is reported that vacuoles found in 3.9% of oocytes at collection, of which 66% had single, 21.3% had double and 12.7% had multiple vacuoles.[6] Similar results were reported by other authors, the studies of whom showed higher oocyte vacuolization rate (5.7 and 12.4% respectively).[7,8]

Rienzi et al[9] reported significantly lower fertilization rates in vacuolated oocytes. It is reported and concluded that the reason for zygotic and embryonic arrest was due to the extremely large cytosolic vacuoles that physically displace the MII spindle from its usual polar position and thus might cause fertilization failure.[10] Similar results were observed and reported that lower normal fertilization rates will occur if vacuoles are up to 14 µm (51.6% for single and 43.8% for multiple vacuoles) and suggest that fertilization cannot be expected if vacuoles are >14 µm.[6]

The aim of this case study was to perform the first analysis of oocytes with a single vacuole and evaluate the potential effects of this oocyte morphological abnormality on intracytoplasmic sperm injection (ICSI) outcomes.

Case Study

The subject aged 27 years was diagnosed with unexplained infertility with normal level of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol 2/3

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day regular cycle and normal anti-Mullerian hormone (AMH) levels. Menstrual cycles were regular every 28 days with moderate flow. She underwent stimulation as per short antagonist protocol with recombinant FSH (rFSH) 225 IU started day 2 of cycle antagonist, which was added day 6 of cycle as per fixed protocol. Injection was administered with 10,000 IU of hCG 35 hours prior to oocyte collection. The male partner aged 30 years showed a relatively normal semen profile for IVF with parameters 135 million/ml, 70% rapid and 5% slow motility and morphology 3% as per krugar criteria. From cohort follicles, 18 oocytes were collected. Three oocytes were identified as metaphase-I, 15 as metaphase-II, and no germinal vesicle (Fig. 1 and Table 1). The oocytes at were observed at 200× magnification identified the presence of single central vacuole in the ooplasm of the all oocytes and thus suitable for injection. Extreme care was taken not to rupture the vacuoles during the injection procedure and sperm was not to enter in the vacuole. A check for cleavage shown after 24 to 36 hours postinjection revealed the presence of two and four-celled embryos (Fig. 2 and Table 2). After 48 to 60 hours of injection and incubation, the embryos show that fertilized oocytes had cleaved, with six to eight-celled embryos (Fig. 3 and Table 2).

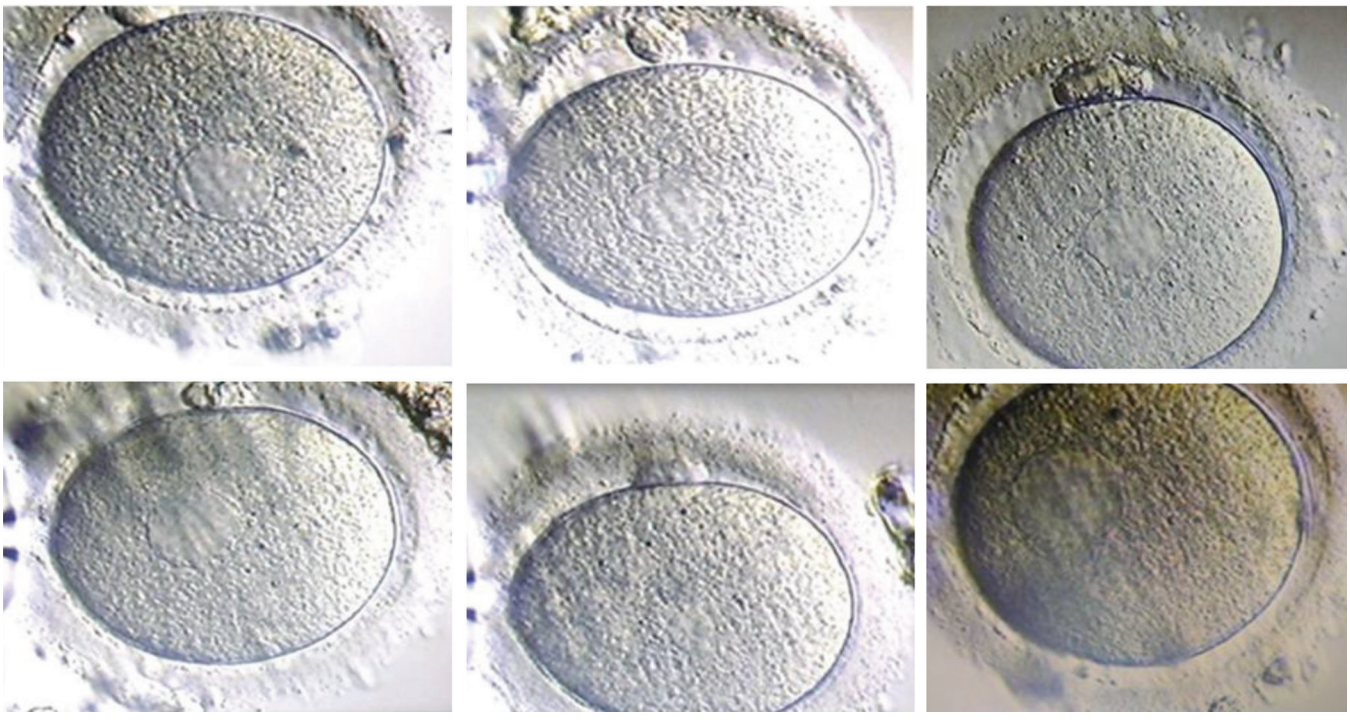

Fig. 1: Vacuolated oocytes

Table 1: Fertilization, cleavage and embryo quality according to cytoplasmic anomalies

		M-II oocytes	*M-I oocytes*	*Fertilized*	*Cleaved*	*Grade "A" embryos*	*Grade "B" embryos*
No. of oocytes	18	15	3	15	15	12	03
Cytoplasmic anomalies	18	15	3	15	15	12	03

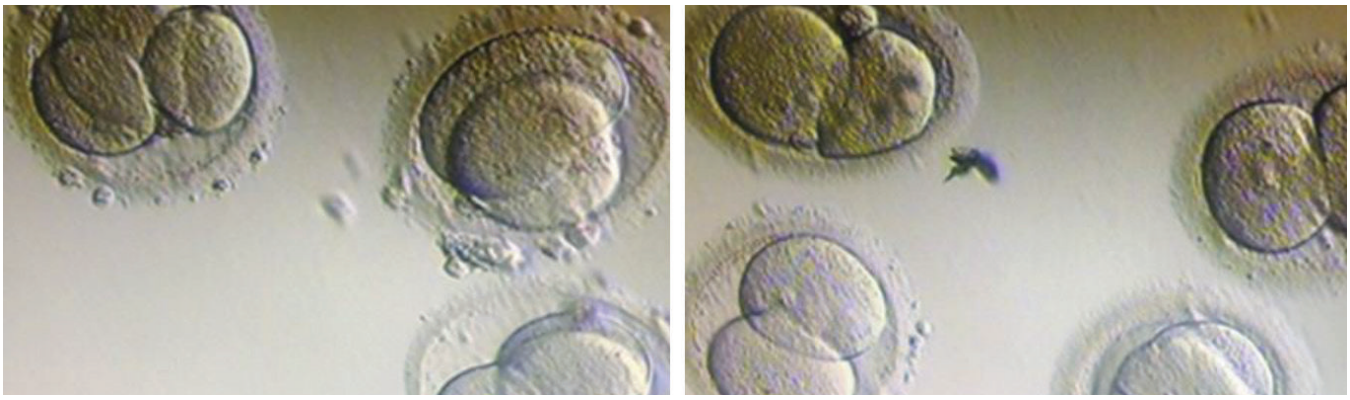

Fig. 2: Twenty-four to 36 hours embryos with 2 to 4 celled

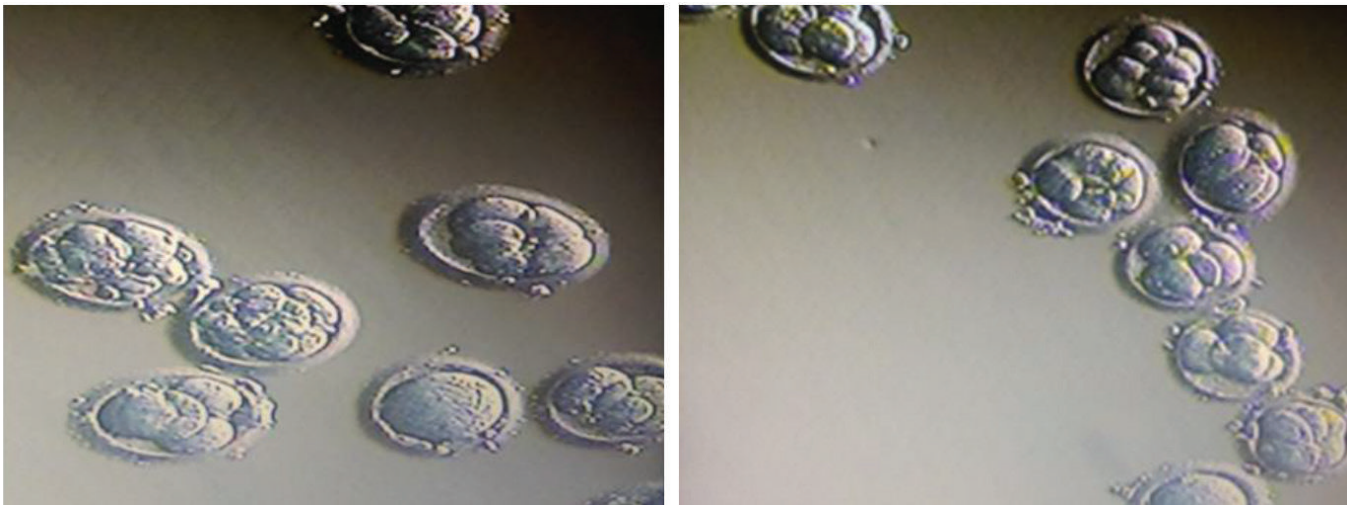

Fig. 3: Fertilized 48 to 60 hours embryos with 6 to 8 celled

Table 2: Subsequent development of oocytes following ICSI after 36 and 60 hours incubation.

Morphology	*No. of oocytes/ embryos*	*36 Hours*	*60 Hours*
Vacuolated	18 oocytes	0	0
2/4 celled embryo	15 embryos	9 (2C)/6 (4C)	0
6/8 celled embryo	15 embryos	0	10 (6C)/5 (8C)

The three embryos were transferred after 60 hours of incubation pregnancy was confirmed after 11 days. Rest of the good quality embryos were cryopreserved.

DISCUSSION

Vacuoles within the cytoplasm are defined as fluid-filled structures that can be more easily noticeable and differ from smooth endoplasmic reticulum (SER) disks. Small vacuoles of 5 to 10 mm in diameter are unlikely to have a biological consequence, whereas large vacuoles 14 µm are associated with fertilization failure. In oocytes that are fertilized, those vacuoles that persist beyond syngamy can interfere with cleavage planes, resulting in a lower blastocyst rate (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011).

The result of the present study shows that vacuolated oocytes can have good fertilization rates. We believe that ours is the one of the first paper to show an ongoing pregnancy when ICSI was done for vacuolated oocytes is a case of unexplained infertility. Similar good fertilization rates were shown by[11,12] with the injection of ''ideal'' oocytes and those displaying vacuoles; however, in one of them, no pregnancy was achieved when an embryo derived from a vacuolated oocyte was transferred.[11] There is no consistent evidence to show that the presence of vacuole affects pregnancy rates.[13]

It is reported and concluded in another case study that the cytoplasmic macro vacuoles with (25 µm) appear to distort oocyte cytoskeletal structure to an extent that physiological processes involved in fertilization and embryogenesis, such as sperm–oocyte signaling, sperm binding, meiotic resumption and embryonic cleavage, are impaired.[14]

The important care that we have taken during intracytoplasmic sperm injection was that sperm should not enter the vacuole also care was taken not to rupture and disturb the vacuole in the oocytes. The results of the present findings in the case study need to be further evaluated. Some more case studies involving vacuolated oocytes give us more data about fertilization rates in vacuolated oocytes and we believe that further research is required. Apart from vacuolated oocytes, sperm morphology such as sperm–oocyte signaling, sperm binding, meiotic resumption, and embryonic cleavage should also be concern for the ideal fertilization of oocytes.

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