Multiple Conjoined Oocytes In A Patient With Polycystic Ovary Syndrome Undergoing In Vitro Fertilization

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Conjoined oocytes are characterized by a follicle containing two oocytes surrounded by a single zona pellucida or their fusion in a zonal region. Gonadotropin stimulation, coupled with PCOS, predisposes to the occurrence of conjoined oocytes. Although a result of developmental accident, conjoined oocytes have the potential to develop into genetically normal embryos and babies. In this paper, the authors describe the aspiration of multiple conjoined oocytes in a single IVF cycle, leading to normal fertilization after selective intracytoplasmic sperm injection (ICSI). Development to blastocyst stage allowed trophectoderm biopsy resulting to a euploid embryo that was eventually transferred, and resulted to a live term birth.

Key words: conjoined oocytes, in vitro fertilization (IVF), intracytoplasmic sperm injection(ICSI)

Introduction

The development of a follicle containing a single haploid female gamete after the resumption of first meiosis is one of the prerequisites to fertilization.[1] A deviation to this rule leads to the phenomenon of binovular follicles or conjoined oocytes.

Conjoined oocytes are characterized by a follicle containing two oocytes surrounded by a single zona pellucida[2,3,4] or their fusion in a zonal region.[2,3] Its occurrence among patients undergoing assisted reproduction is rather limited.[5] In a literature review conducted by Rosenbusch and Hancke[2] in 2012, there were only 18 reported cases of conjoined oocytes retrieved in patients undergoing ovarian stimulation for in vitro fertilization (IVF).

In this paper, the authors describe the aspiration of multiple conjoined oocytes in a single IVF cycle, leading to normal fertilization after selective intracytoplasmic sperm injection (ICSI). Development to blastocyst stage allowed trophectoderm biopsy resulting to a euploid embryo that was eventually transferred, and resulted to a live term birth.

The Case

Patient Profile

The index case is a 36-year-old G0 and her 42-year-old husband for 5 years. The couple consulted at the Center for Advanced Reproductive Medicine and Infertility, St. Luke's Medical Center – Global City for their infertility. She is a known case of polycystic ovary syndrome (PCOS) and has previously undergone laparoscopic management of her bilateral endometriomas.

The couple had two failed cycles of ovulation induction with intrauterine insemination. Initial laboratory workup results are presented in Table 1. The couples' infectious panel (HBsAg, RPR, HepC, HIV-1 and 2) were all nonreactive. Semen analysis of the male partner was normozoospermia.

Controlled Ovarian Stimulation

Ovarian stimulation was carried out using the gonadotropin-releasing hormone (GnRH) antag-

Table 1. Initial laboratory workup.

Female partner		Male partner: semen analysis	
AMH	10.55ng/mL	Sperm concentration	78 x 10^6/mL
Estradiol	29.11 pg/mL	Total sperm count	161.5 x10^6/ejaculate
Prolactin	13.72 ng/mL	Total motility	73%
TSH	1.049	Total motile count	117.9 x10^6
Vitamin D, total	22.54 ng/mL	Progressive motility	63%
		Normal forms	5%

onist protocol. Recombinant follicle stimulating hormone (Gonal-F, Merck Serono, Switzerland) with combination of recombinant follicle stimulating hormone and recombinant luteinizing hormone (Pergoveris, Merck Serono, Switzerland) were started on the second day of the menstrual cycle. Pituitary suppression was achieved by administration of GnRH antagonist (Cetrotide 0.25mg, Merck Serono, Switzerland), starting on day 6 of stimulation. Ovulation trigger with GnRH agonist, Decapeptyl 0.2mg (Ferring, Switzerland) was administered on day 10 of stimulation, to avoid the development of ovarian hyperstimulation syndrome.

Oocyte Retrieval and Intracytoplasmic Sperm Injection (ICSI)

Oocyte retrieval under intravenous anesthesia was scheduled 36 hours after the ovulation trigger. Ultrasound-guided ovum pickup was carried out using a transvaginal probe, attached to a gauge 16 double-lumen needle, connected to a collecting tube. Each follicle was punctured by the needle, and the follicular fluid was aspirated at 120 mmHg. The collecting tubes were immediately sent to the adjacent laboratory for evaluation of the presence of cumulus-oocyte complexes (COCs). There were 25 COCs retrieved and were immediately denuded: 16 metaphase II (MII), 5 germinal vesicles (GV) and 4 conjoined oocytes, each containing MII and GV (Figure 1). ICSI was performed on all mature oocytes, which were then washed and incubated at 6% CO_2, 5% O_2 and 89% N_2.

Fertilization and Oocyte Development

Injected oocytes were assessed for the presence of polar bodies and formation of two pronuclei (2PN) after 18 hours. Normal fertilization of 13 oocytes was noted, two of which coming from the conjoined oocytes (Figure 2). The other two sets of conjoined oocytes remained unfertilized. Three days after, all fertilized embryos continued to develop into 6-cell to 10-cell cleavage stage embryos, including the conjoined oocytes at 8-cell grade 2 and 8-cell grade 2+ (Figure 3). Eight oocytes eventually developed to the blastocyst stage, including the 2 conjoined oocytes at 3BB and 3CB (Figure 3). All surviving blastocysts were biopsied and samples were sent for genetic analysis by Next Generation Sequencing (NGS, Bangkok, Thailand). Preimplantation genetic screening (PGS) showed euploid 3BB,XX and aneuploid 3CB,XY embryos arising from the conjoined oocytes; and 2 euploid (4BB,XY and 4BA,XY), 2 aneuploid (3BB,XY and 5BC,XX) blastocysts and 2 embryos with DNA degradation (both 5CC) arising from uniovular folllcles.

Embryo Transfer and Pregnancy Outcome

Frozen embryo transfer was performed two cycles after the oocyte retrieval. After informed consent, the couple requested to transfer two embryos, choosing one of the euploid embryos from the conjoined oocytes. The GV oocyte conjoined to the euploid XX blastocyst (3BB) was removed by assisted hatching and the remaining blastocyst

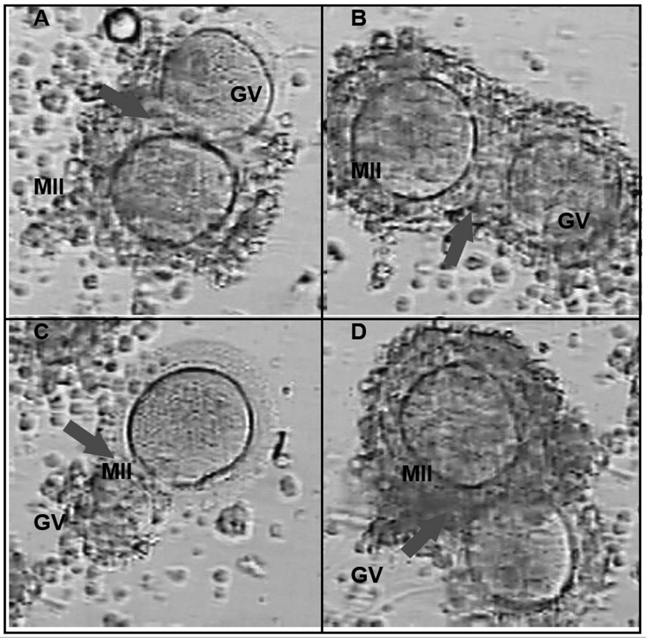

Figure 1. Conjoined oocytes. Four sets of conjoined oocytes were harvested, each containing a larger metaphase II and a smaller germinal vesicle (GV). The arrows depict the connected region of the zona pellucida. (A) and (C) share the same zona pellucida, while (B) and (D) are conjoined oocytes that have fused zona pellucida.

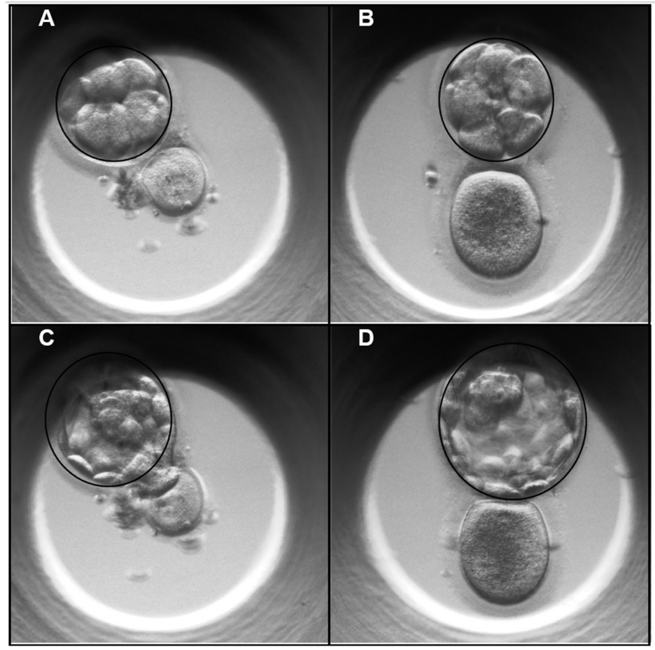

Figure 3. Cleavage and blastocyst stages (enclosed in a broken circle). The two sets of conjoined oocytes developed to cleavage stage embryos three days after insemination: 8-cell grade 2 (A) and 8-cell grade 2+ (B). Five days after sperm injection, the oocytes developed into blastocysts stage 3BB (C) and stage 3CB (D).

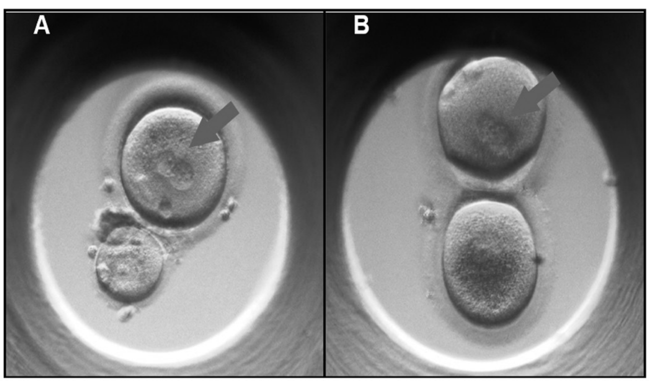

Figure 2. Fertilized conjoined oocytes. Two sets of conjoined oocytes completed fertilization. The arrows depict the fertilized conjoined oocyte, as demonstrated by two pronuclei (2PN) while the accompanying oocyte for each set (A, B) arrested at GV.

was transferred together with a euploid XY embryo (4BB). Fourteen days after the transfer, serum beta hCG was 1900 mIU/mL, indicative of an early pregnancy.

Patient eventually delivered to healthy twins by cesarean section at 37 weeks. The baby girl, arising from the conjoined oocyte, weighed 2300g while the baby boy weighed 2700g.

Discussion

Ovarian follicles containing more than one oocyte has been documented in mammalian species as early as 1926.[5] Its occurrence has been described in the immature human ovary, and is thought to rarely persist in adulthood due to atresia in late fetal and early neonatal life.[6,7,8,9,10] In 1983, the recovery of binovular follicle in a woman undergoing ART was first reported.[11]

The theories to explain the existence of conjoined oocytes are as a developmental accident[4,5,11,12] and in abnormal meiotic division. In the first, and probably the more plausible reason for the formation of binovular follicles, there is failure of separation of two adjacent germ cells. In early folliculogenesis, the granulosa cells or connective tissues fail to completely surround each individual oocyte, leading to the apposition of adjacent cells.[2,3,4,6,7,10,11] For this reason, the fusion of zona depends on the distance between neighboring germ cells: 1) two oocytes sharing a common and intact zona, or 2) two oocytes with individual zona but joined in a defined region.[2,3,4,12]

In the abnormal meiotic division theory, there is encapsulation of syncytial cells in the sex cord that fail to separate after the mitotic phase of oogenesis. Intercellular bridges are then established to serve as a direct communication between cytoplasm of the formed conjoined oocyte.[6,8,10] Following this concept, conjoined oocytes that develop through this process are expected to have equal and synchronous development.

In this report, there were four sets of conjoined oocytes retrieved: two sets sharing a common and intact zona; and two sets with individual zona. All pairs consist of a larger MII with a smaller GV oocyte, displaying a maturational asynchrony, that is similar with the finding of Ben-Rafael, et al.[10] This discrepancy in the state of each egg exhibit their individual potential to respond to stimuli to either grow or remain retarded.[5] Thus, the morphological characteristics of all the conjoined oocytes in this report were in support of the first hypothesis.

Several factors were linked to the occurrence of conjoined oocytes such as ovarian stimulation[4,5,6,78,10], PCOS[1,4,12,13,14] and exposure to diethylstilbestrol.[1]

Exogenous gonadotropin administration has been commonly cited as a key factor in the appearance of conjoined oocytes. Papadaki[8] postulated that high levels of gonadotropins, such as in ovarian stimulation in IVF, stimulate proteolytic enzyme activity resulting to the degradation of the ground substance between closely adjacent follicles and leading to their fusion. Furthermore, the authors believe that the presence of polycystic ovaries further contributed to the appearance of conjoined oocytes, not only due to increased number of follicles[4,12], but also due to varying maturational status of oocytes. This is in support of the proposition by Yano, et al.[4], where PCOS patients exposed to exogenous gonadotropin for ovarian stimulation, increased their chances of having conjoined oocytes.

In this report, there were two out of the four sets of conjoined oocytes retrieved that achieved successful fertilization after ICSI. Both cleaved and continued development to blastocyst stage. Table 2 summarizes the reported cases of conjoined oocytes in the literature. To date, there is a total of 32 documented cases, including four from the present report. Among these, 56% (18 cases) had at least one of the conjoined oocytes fertilized and 89% (16 out of 18) of the fertilized oocytes proceeded to cleavage and blastocyst stages. The high fertilization and development rates prove that any given conjoined oocyte in a good maturational stage, is as equally capable of fertilization and development as that of an oocyte arising from uniovular follicles.

Genetic analysis of conjoined oocytes was first carried out by Safran, et al.[6] via fluorescence in situ hybridization (FISH). They observed a diploid chromosomal constitution of both immature germinal vesicle, as well as the embryo that developed after ICSI. Moreover, Cummins, et al.[3] performed a PGS concomitantly with laser hatching in one of the two conjoined oocytes they retrieved, which resulted to a euploid embryo. In the present report, two blastocysts arising from conjoined oocytes were subjected to PGS, which resulted to one aneuploid and one euploid embryos. These results showed that a genetically normal embryo can be achieved from those arising from conjoined oocytes.

Several studies highlighted the possible implications of transferring embryos arising from conjoined oocytes. This include dizygotic twinning [1,9,10,11], tri-/tetraploidy[1,10] and chimerism.[1,10,1 1] In contrast, successful uncomplicated live births have been likewise reported. In the literature reviewed in Table 2, among the 7 embryo transfers performed, 3 resulted in successful pregnancies, including this case report.

In the first case reported by Cummins, et al.[3], conjoined oocytes that were retrieved consisted of an MII and a GV oocyte. ICSI was performed in the mature oocyte resulting to fertilization. The unfertilized immature oocyte was removed concomitantly with laser hatching on Day 3 and a PGS was performed resulting to a euploid embryo. Among the embryos arising from uniovular follicles, 3 were noted to be aneuploid on PGS and two were discarded due to poor quality. The blastocyst arising from the conjoined oocyte was transferred on Day 5 resulting to pregnancy and a livebirth of a 37-week baby girl, weighing 3200g, via cesarean section.[3]

Yano, et al.[4] retrieved 2 conjoined oocytes: one pair with both MII and the other with both MI. Only the first pair was fertilized and developed to blastocyst stage. A total of 3 blastocysts were cryopreserved, including the one arising from the conjoined oocyte. Upon thawing, the two blastocysts from uniovular follicles were found to be of poor quality, leading to the transfer of a blastocyst

Table 2. Summary of reported cases of conjoined oocytes.

Zona structure	Maturity of gametes		Fertilization		Further development	References
	Oocyte 1	Oocyte 2	Oocyte 1	Oocyte 2		
Oocytes with own, connected zona	MII	MII	2PN	2PN	Cleavage and ET of one embryo after mechanical separation, no pregnancy	Zeilmaker GH et al
Oocytes share an intact single layer	?	MII	--	2PN	Cleavage and ET, no pregnancy	Ben-Rafael Z et al
Oocytes share an intact single layer	GV	MII	--	--	Fixed for analysis	Fishel S et al
Oocytes share an intact single layer	GV	MII	--	2PN	Frozen for analysis at blastocyst stage	Hartshorne GM et al
Oocytes share an intact single layer	GV	GV	--	--	--	Ron-El R et al
Oocytes share an intact single layer	GV	MII	--	--	--	
Oocytes share an intact single layer	GV	MII	--	2PN	Cleavage and ET, no pregnancy	
Thin or not present	GV	MII	--	3PN	--	
Oocytes share an intact single layer	GV	GV	--	--	--	
Oocytes share an intact single layer	GV	MI	--	2PN	Cleavage, no ET	Safran et al
Oocytes share an intact single layer	GV	MI	--	2PN	Fixed for analysis after cleavage	
Oocytes share an intact single layer	GV	MI	?	?	?	Veeck LL
Oocytes share an intact single layer	GV	MII	--	2PN	Cleavage to blastocyst, no ET	Vicdan K et al
Oocytes with own, connected zona	GV	MII	?	?	?	Kousehlar M et al
Thin or not present	GV	?	?	?	?	
Discontinuous with a breech	GV	GV	--	--	--	Rosenbusch B and Hancke K
Oocytes share an intact single layer	GV	MII	--	--	--	
Oocytes with own, connected zona	GV	MII	--	2PN	Cleavage and ET, no pregnancy	
Oocytes with own, connected zona	MII	MII	2PN	2PN	No embryo	Asimakopoulos et al
Oocytes with own, connected zona	MII	GV	2PN	--	Cleavage, Day 3	
Oocytes share an intact single layer	MII	MII	--	--	--	
Oocytes with own, connected zona	MII	MII	2PN	--	Cleavage, Day 3	
Oocytes with own, connected zona	MII	GV	2PN	--	Cleavage, no ET	Tanaka et al
Oocytes with own, connected zona	MII	GV	--	--	--	
Oocytes with own, connected zona	MII	GV	2PN	--	Blastocyst and ET, (+) pregnancy	Cummins et al
Oocytes with own, connected zona	MII	MII	2PN	--	Blastocyst, mechanical separation then ET, (+) pregnancy	Yano et al
Oocytes with own, connected zona	MI	MI	--	--	--	
Oocytes with own, connected zona	MII	MI	2PN	--	Blastocyst, no ET	
Oocytes share an intact single layer	MII	GV	--	--	--	Present report
Oocytes with own, connected zona	MII	GV	--	--	--	
Oocytes share an intact single layer	MII	GV	2PN	--	Blastocyst, no ET	
Oocytes with own, connected zona	MII	GV	2PN	--	Blastocyst and ET, (+) pregnancy	

**shaded area lifted from the report of Rosenbusch B and Hancke K in 2012*
**MII – metaphase II, MI – metaphase I, GV – germinal vesicle, PN – pronuclei, ET – embryo transfer*

arising from the conjoined oocyte after laser manipulation. This resulted to a pregnancy and delivery of a 38-week-old male, weighing 3510g. The boy was assessed to be healthy at 5 years of age.[4]

In the current study, the GV oocyte conjoined to the euploid XX blastocyst (stage 3BB) was removed and transferred together with a euploid XY embryo at stage 4BB, which both resulted to pregnancy. Cesarean section for the twin pregnancy was performed at 37 weeks with the delivery of healthy term babies including the baby girl from the conjoined oocytes. These studies are evidences that although a result of developmental accident, conjoined oocytes have the potential to develop into genetically normal embryos and normal babies.

Although these reports are promising, caution should still be undertaken when transferring embryos arising from conjoined oocytes. Additional information as to the long-term outcomes is still needed, and these should be thoroughly disclosed to the patient.

Conclusion

Gonadotropin stimulation, coupled with PCOS, predisposes to the occurrence of conjoined oocytes. Although a result of developmental accident, conjoined oocytes have the potential to develop into genetically normal embryos and babies. This is the third reported case of a conjoined oocyte resulting to a live birth, and probably the highest number of conjoined oocytes retrieved in a single IVF cycle.

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