Repeated collection of conjoined oocytes from a patient with polycystic ovary syndrome, resulting in one successful live birth from frozen thawed blastocyst transfer: a case report

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Abstract Few cases have been reported in which the aspiration of a single follicle led to the recovery of two conjoined oocytes surrounded by a single zona pellucida. This report describes a successful embryo transfer with subsequent live birth derived from conjoined oocytes, and a later pair of conjoined oocytes in the same patient. After oocyte retrieval from a patient with polycystic ovary syndrome, two pairs of conjoined oocytes were collected. One oocyte was fertilized using in vitro fertilization (IVF) and developed to the blastocyst stage. This blastocyst was cryopreserved and later transferred to the uterus after separating the unfertilized conjoined oocyte. A successful pregnancy and healthy live birth was achieved. Two years later, the patient returned for a second IVF; one pair of conjoined oocytes was detected. One of the pair was fertilized and developed to a blastocyst, but was not transferred. We demonstrate that selective fertilization of a mature oocyte from conjoined oocytes by IVF can lead to the development of a blastocyst and subsequent pregnancy and live birth. To our knowledge, this is the second case report of successful live birth from conjoined oocytes. It may be the first case of repeated fertile conjoined oocytes from the same patient.

Keywords Conjoined oocytes · Binovular follicle · In vitro fertilization · Polycystic ovary syndrome · Oocyte retrieval

Introduction

In the literature of assisted reproductive technology (ART), few cases have been reported in which aspiration of a single follicle following ovulation induction yielded two conjoined oocytes surrounded by a single zona pellucida (Fig. 1). A binovular cumulus mass containing two oocytes, each surrounded by a separate zona pellucida, was first reported by Zeilmaker et al., followed by a few other case reports [1–3]. Histological studies of multiovular ovarian follicles have been described in various mammalian species, including humans [4, 5]. Two convincing hypotheses are suggested explaining the existence of binovular follicles. In the first, a binovular follicle is the result of a developmental accident, wherein connective tissue development fails to separate closely proximate oocytes [1]. The second hypothesis suggests that this phenomenon of two oocytes within a single zona pellucida is generated through an abnormal meiotic division [2]. Their reported incidence, 0.1%, demonstrates their rarity [3]. The fact that in histologic specimens the incidence of such binovular follicles was between 5 and 13% (50 to 130 times greater than in oocyte retrieval), might be due to malresponse of binovular primordial follicles to ovarian hyperstimulation [3–5]. Although the frequency of conjoined oocytes is extremely low in natural cycles, it has been suggested that

Fig. 1 Illustration of binovular follicle

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Fig. 2 Polycystic ovaries. The polycystic pattern was defined in both ovaries using transvaginal ultrasonography by the presence of 10 or more cysts measuring 2–9 mm at the first visit to our clinic. Patient was diagnosed with polycystic ovary syndrome (PCOS)

ovarian stimulation by in vitro fertilization (IVF) might increase the observed incidence of such follicles in the periovulatory stage [6].

Two cases have been described in which intracytoplasmic sperm injection (ICSI) of a mature oocyte from conjoined oocytes allowed fertilization and development of a chromosomally balanced cleavage-stage embryo [7]. Embryos developed from conjoined oocytes are chromosomally normal and have developed to the blastocyst stage [8, 9]. In a recent four-case analysis, it was concluded that mature oocytes in conjoined oocytes can be fertilized, but have limited developmental potential and should be considered abnormal [10].

However, pregnancy following transfer of a blastocyst obtained from a conjoined oocyte has been reported, by Cummins et al., who reported a live birth from a conjoined oocyte, involving blastocyst transfer of a genetically normal embryo [11]. In the present case report, we describe the successful transfer of a post-thawed cryopreserved blastocyst, derived from one of a pair of conjoined oocytes, resulting in a live birth. Two years after delivery, the patient again visited our clinic and expressed the desire for a second child. A standard IVF program was again planned. Five cumulus-oocyte complexes (COCs) were collected, including one pair of conjoined oocytes. To our knowledge, this is the second case report of a successful live birth from a conjoined oocyte. It

may be the first case of repeated collection of fertile conjoined oocytes in the same patient.

Case presentation

Patient profile

A 30-year-old female (G0P0) presented at the Yano Maternity Clinic IVF Center in September 2010, and expressed the desire to have a baby. Since her menarche at age 12 years, she had oligomenorrheic cycles and intermittent hormone replacement therapy (HRT). She was slender (height, 157 cm; weight, 46 kg) and physically and intellectually normal. Anatomical structures of the reproductive tract were within normal ranges and she had no underlying disorder such as infectious disease or immunological dysfunction.

Blood samples of the patient revealed endocrine abnormality, with high levels of serum luteinizing hormone (LH) (25.4 mIU/mL), progesterone (P) (40.27 ng/mL), and anti-müllerian hormone (AMH) (35.9 ng/mL). A normal level of follicle-stimulating hormone (FSH) (6.0 mIU/mL) and a low level of estradiol (E2) (63.0 pg/mL) were revealed. Additional blood testing included prolactin (PRL) (18.0 ng/mL), thyroid-stimulating hormone (TSH) (0.92 μ IU/mL), free thyroxine

Fig. 3 The connected region of the zona pellucida is indicated by black arrows. **a** After insemination, one conjoined oocyte was shown to be fertilized, having two pronuclei (2PN) and another appeared to be

unfertilized at the metaphase II stage (M II). **b** Another conjoined oocyte was not fertilized. Both oocytes were in metaphase I stage (M I)

Fig. 4 **a** One oocyte fertilized of the conjoined pair, cleaved to eight-cell stage on day 3, and **b** same oocyte developed to the blastocyst stage on day 5

(FT4) (1.27 ng/dL), insulin (3.0 μ IU/mL), hemoglobin A1c (HbA1c) (4.9%), and negative anti-nuclear antibody (ANA) (1:40 >), all within normal ranges.

The polycystic pattern in both ovaries was identified by the presence of 10 or more cysts measuring 2 ~ 9 mm in diameter on transvaginal ultrasound (Fig. 2). She was diagnosed with polycystic ovary syndrome (PCOS). Her 31-year-old husband was healthy and his semen was normal; sperm concentration was 41×10^6 /mL, and the motility rate was 60.0% with normal morphology.

Ovulation induction

Clomiphene citrate (CC) was used for ovulation induction three times but failed to induce ovulation. Since she was CC resistant, ovulation induction with exogenous gonadotropin therapy was recommended. Follicular growth was not induced with injection of low-dose FSH (Follistim; MSD) at a beginning dose of 50 IU/day. Since the ovaries did not respond, the dosage quantity and frequency were increased gradually; a large number of follicles developed. To avoid multiple pregnancy and ovarian hyperstimulation syndrome (OHSS), IVF, and embryo transfer (ET) were recommended.

IVF protocol

Our protocol has been previously described [12]. Ovulation induction was carried out with combination of gonadotropin-

releasing hormone agonist (Suprecur; Mochida Pharmaceutical) and the injection of FSH and human menopausal gonadotropin (HMGFuji; Fuji Pharma). Oocytes were retrieved 35.5 h after human chorionic gonadotropin (hCG) administration (5000 IU; HCG Mochida; Mochida Pharmaceutical) under transvaginal ultrasound guidance.

Conjoined oocytes

Six COCs were collected. Since the husband's semen was normal, standard IVF was carried out on the six COCs. Nineteen hours later, two pairs of conjoined oocytes were found within two of the COCs. Both oocytes appeared mature and of equal size. Following insemination, one of the conjoined oocytes was found to be fertilized, having two pronuclei, whereas its partner appeared unfertilized at metaphase stage II (M II) (Fig. 3a). Neither oocyte of the other conjoined oocyte pairs was fertilized, both showing metaphase stage I (M I) (Fig. 3b). Each conjoined oocyte of both pairs was surrounded by its individual zona pellucida (ZP) and connected by a thin common isthmus of ZP, the two ZPs appeared to be fused (Fig. 3). By the 3rd day after insemination, the fertilized oocyte had cleaved to the eight-cell stage (Fig. 4a), and it reached the blastocyst stage on day 5 (Fig. 4b). The unfertilized conjoined oocyte remained in the germinal vesicle (GV) stage (Fig. 4). The four remaining COCs contained three morphologically normal mature oocytes and one immature oocyte. Regular formation of two pronuclei

Fig. 5 Dissection to the zona pellucida (ZP) of unfertilized oocyte. The ZP of the unfertilized oocyte of the conjoined-oocyte complex was manipulated to dissect the whole ZP circumference using diode laser. **a** The

ZP of unfertilized oocyte is shown in the dotted line. **b** Embryo just before transfer: After removal of the unfertilized oocyte, laser-assisted hatching (LAH) was performed (site of LAH indicated by arrows)

Fig. 6 Repeated conjoined oocytes in the same patient. One oocyte was mature while the other was less than one-fourth normal size. **a** Day 1: newly fertilized. **b** Day 3: eight-cell stage. **c** Day 5: blastocyst stage. **d** Day 6: hatched blastocyst

occurred in the three normal oocytes; the immature oocyte was not fertilized. All fertilized oocytes were cultured in Quinn's Advantage Series protein plus medium (SAGE In Vitro Fertilization, USA) until day 5. On day 5, three viable expanded blastocysts, including the one developed from the conjoined oocytes, were obtained.

Cryopreservation

The three blastocysts were cryopreserved using the vitrification method (Cryotop Safety Kit; Kitazato Corporation, Japan) [13]. After thawing all the blastocysts, the two derived from normal oocytes were judged to be poor quality and unsuitable for transfer. We therefore decided to transfer the blastocyst from the conjoined oocyte.

Laser manipulation

Laser was used to dissect the connection site of the ZP of the conjoined oocytes. The ZP of the unfertilized oocyte of the conjoined-oocyte complex was manipulated to dissect the whole ZP circumference using a 1.48- μ m infrared diode laser (OCTAX Laser Shot System: MTG, Germany) and the unfertilized oocyte removed using a pipette (Fig. 5a). Laser-assisted hatching (LAH) was then performed to thin the ZP by the previously described procedure of zona thinning [14] (Fig. 5b).

Embryo transfer

Embryo transfer was successfully performed at the appropriate point in a hormone replacement cycle (Fig. 5b). Singleton

pregnancy was established, and a normal 3510 g male infant was delivered at 38 weeks' gestation in November 2011. More than 5 years have passed since this birth, and the child has grown up healthy.

Repeated conjoined oocytes

Two years after delivery, the patient visited our clinic seeking a second baby. A similar IVF program was planned and performed. Five COCs were retrieved. Among five COCs, one pair of conjoined oocytes was detected. One oocyte was mature, whereas the connected oocyte was less than one-fifth the normal size at M I stage (Fig. 6a–d). Three mature oocytes, including the mature conjoined oocyte, were fertilized and developed to blastocysts. After thawing of all these blastocysts, the blastocyst derived from conjoined oocytes was not transferred to the uterus because another, better-quality blastocyst from a normal oocyte was judged to be suitable for transfer. This IVF procedure did not succeed in producing a second pregnancy.

Discussion

The probable mechanism for the formation of conjoined oocytes was set forth by Hartman [15] who proposed a failure of separation of two individual germ cells early in folliculogenesis. Two oocytes surrounded by a single zona pellucida are so closely apposed that they fail to become individually surrounded by granulosa cells. Thus, the zona pellucida becomes a continuous layer surrounding both oocytes during folliculogenesis [1]. Various patterns of zonal fusion

have been observed, such as two oocytes sharing a common intact ZP, or two oocytes each with an individual ZP but joined in a defined region [10]. Safran et al. [7] analyzed a pair of conjoined oocytes by fluorescence in situ hybridization, providing evidence that each of the oocytes represented an individual gamete with a chromosomal constitution corresponding to its stage of maturity. In a review of the literature, a total of 18 reports of conjoined oocytes were described. Of these reported cases, 11 of 18 contained an M II-phase oocyte joined to a GV, three contained two GVs, one contained an M I oocyte and a GV, and one contained two M II oocytes [9]. Turkalj et al. [10] detailed four cases in women aged 22 to 33 years old. They concluded that mature oocytes contained in conjoined-oocyte complexes can be fertilized, but have limited developmental potential and should be considered abnormal.

Recently, Cummins et al. [11] reported that a healthy female baby was achieved by embryo transfer of a genetically normal blastocyst, performed after removal of the immature oocyte from the conjoined oocyte on day 3. The embryo was screened with array comparative genomic hybridization (a-CGH) and the euploid embryo was transferred as a hatching blastocyst on day 5. However, there are very limited data available in the literature on conjoined oocytes from binovular follicles in humans. When fertilization and embryo development do occur, it is possible the degenerated unfertilized oocyte could negatively affect the developing embryo. Thus, it is assumed to be preferable to transfer other, normally developing embryos, rather than those derived from conjoined oocytes. In our case, after removal of the degenerated unfertilized oocyte of a conjoined-oocyte complex, a diode laser was used to thin the ZP prior to blastocyst transfer. Such use of a 1.48- μ m infrared diode laser has been shown to have no adverse effect on subsequent embryo development [16].

This may be the first report of conjoined oocytes obtained repeatedly in the same patient. It is reasonable to assume that, for a patient with PCOS, the exogenous gonadotrophins administered during ovarian stimulations increase the incidence of abnormal follicles, including conjoined oocytes [3]. Turkalj et al. [10] described four cases that supported this notion; the common element was numerous follicles resulting from pronounced response to ovarian stimulation. This could cause increased likelihood of repeated conjoined-oocyte collection in a PCOS patient, as in our case.

Conclusions

We recovered conjoined oocytes in our practice of ART and demonstrated that a mature oocyte from a conjoined-oocyte complex can lead to the development of a blastocyst by standard IVF, and subsequently achieve pregnancy and live birth. It is likely that the phenomenon of conjoined oocytes occurs in

many laboratories, and this report provides evidences that such conjoined oocytes can be used to produce viable pregnancies. To our knowledge, this is the second case report of a successful live birth from conjoined oocytes. It may also be the first report of repeated fertile conjoined-oocyte collection from the same patient.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Human rights statements and informed consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and the Helsinki Declaration of 1964 and its later amendments. Informed consent was obtained from all patients included in the study.

Animal studies This article does not contain any studies with animal subjects performed by any of the authors.

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