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ASSISTED REPRODUCTION TECHNOLOGIES

Live birth from a blastocyst derived from a conjoined oocyte in a frozen embryo transfer cycle: a case report and a literature review

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Abstract

The significance of conjoined oocytes in the clinical in vitro fertilization (IVF) laboratory setting has been questionable due to the extremely limited data available. This issue is discussed by presenting one case for conjoined oocyte observed in the program of the assisted reproduction and by including a review of corresponding literature. This report describes a successful clinical pregnancy with subsequent live birth from a conjoined oocyte. To our knowledge, there are only three reported cases of successful live birth from conjoined oocytes, but this is the first case of live birth from a blastocyst derived from a conjoined oocyte fertilized using intracytoplasmic sperm injection (ICSI) in a frozen embryo transfer cycle. Moreover, this study reports the first time that live birth of a conjoined oocyte is achieved without removing the degenerated immature oocyte prior to transfer. It demonstrates that the degenerated immature oocyte has no adverse effect on subsequent embryo development and pregnancy outcome. In addition, we reviewed the literature to evaluate the origin, incidence, safety, and significance of conjoined oocytes in reproductive health. We further confirm previous reports that demonstrate that a mature oocyte from conjoined-oocyte complexes can be fertilized by standard IVF or ICSI and lead to the development of a blastocyst, subsequent pregnancy, and live birth.

Keywords Conjoined oocyte · Binovular follicle · Intracytoplasmic sperm injection · Frozen embryo transfer cycle

Introduction

Polyovular follicles have been observed by histological studies of the ovaries and laparoscopic oocyte retrieval procedures during assisted reproductive treatments [1–4]. At this time, the most reliable criterion of polyovular follicle is the inclusion of two oocytes within a common zona pellucida or their fusion in the zonal region [5]. However, the significance of conjoined oocytes in the assisted reproductive technology (ART) has been of question due to the extremely limited data in the literature. Therefore, it is extremely probable that these conjoined oocytes retrieved during the ART treatments would not be used for attempts at fertilization and subsequent embryo development. Moreover, there is a few chance for embryo transfer derived from a conjoined oocyte due to having the other normally developing embryos. Conjoined oocytes retrieved after ovarian stimulation were described in various literature and books [6–9]. In review of Table 1, the published cases have shown that another fifty case descriptions of conjoined oocytes were found [5–8, 10–22]. To our knowledge, there are only three reported cases of successful live birth from conjoined oocytes, two of which were achieved by fresh embryo transfer after intracytoplasmic sperm injection (ICSI) [10, 15]. One was achieved by frozen blastocyst transfer following fertilization using in vitro fertilization (IVF) [16]. In the present study, we report for the first time live birth of a blastocyst derived from a conjoined oocyte after ICSI in a frozen embryo transfer cycle. Furthermore, we reviewed the literature to evaluate the origin, incidence, safety, and significance of conjoined oocytes in reproductive health.

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Table 1 Cases of conjoined oocytes in previous and current studies

Maturity of gametes		Zona structure at junction	Fertilization		Fertilization method	Further development	References
Oocyte 1	Oocyte 2		Oocyte 1	Oocyte 2			
MII[a]	GV	Own but connected zona	2PN	–	ICSI	Blastocyst and ET, **live birth**	[15]
MII[a]	MII[a]	Common intact single layer	2PN	–	IVF	Blastocyst, frozen-thawed and ET, **live birth**	[16]
MII[a]	MII[a]	Own but connected zona	2PN	2PN	ICSI	Blastocyst and ET, **live birth**	[10]
MII[a]	?	A modified, discontinuous structure(see text)	2PN	–	ICSI	Blastocyst, frozen-thawed and ET, **live birth**	Present report
MII[a]	MII[a]	Own but connected zona	2PN	2PN	IVF	Cleavage and ET, **no pregnancy**	[11]
MII[a]	?	Common intact single layer	2PN	–	IVF	Cleavage and ET, **no pregnancy**	[12]
MII[a]	GV	Common intact single layer	2PN	–	IVF	Cleavage and ET, **no pregnancy**	[5]
MII[a]	GV	Own but connected zona	2PN	–	ICSI	Cleavage and ET, **no pregnancy**	[7]
MI	MI	Common intact single layer	–	–	–	–	[16]
MII[a]	MI	Common intact single layer	2PN	–	IVF	Blastocyst, cryopreservation	
MII[a]	GV	Common intact single layer	2PN	–	ICSI	Blastocyst, cryopreservation	[17]
MII[a]	GV	Own but connected zona	2PN	–	ICSI	Cleavage, cryopreservation	[13]
MI	Atresia	Own but connected zona	–	–	–	–	
MII[a]	GV	Common intact single layer	2PN	–	IVF	Frozen for analysis at blastocyst stage	[22]
GV	GV	Common intact single layer	–	–	–	–	[5]
MII	GV	Common intact single layer	–	–	–	–	
MII[a]	GV	Thin or not present	3PN	–	IVF	–	
GV	GV	Common intact single layer	–	–	–		
MII[a]	GV	Common intact single layer	2PN	–	ICSI	Cleavage, no ET	[18]
MII[a]	GV	Common intact single layer	2PN	–	ICSI	Fixed for analysis after cleavage	
MII	GV	Common intact single layer	?	?	?	?	[8]
MII[a]	GV	Common intact single layer	2PN	–	ICSI	Blastocyst, no ET	[19]
MII	GV	Own but connected zona	?	?	?	?	[20]
GV	?	Thin or not present	?	?	?	?	
GV	GV	A discontinuous structure	–	–	–	–	[7]
MII	GV	Common intact single layer	–	–	–	–	
MII[a]	MII[a]	Common intact single layer	2PN	2PN	ICSI	Fertilized but did not developed into embryos	[14]
MII[a]	GV	Common intact single layer	2PN	–	ICSI	Cleavage, no ET	
MII	MII	Within a common ZP	–	–	–	–	
MII[a]	MI	Common intact single layer	2PN	–	ICSI	Cleavage, no ET	
MII[a] (16)	GV (16)	Own but connected zona; Common intact single layer; Within a common ZP	2PN (6)	–	IVF	Cleavage (4), arrest; Blastocyst (2), cryopreservation	[21]
MI (2)	GV (2)		–	–	–	–	
MI	MI		–	–	–	–	
GV	GV		–	–	–	–	
MII	GV	Common intact single layer	–	–	–	Fixed for analysis	[6]

[a]Mature oocyte was attempted for fertilization. ?—not indicated. *GV*, germinal vesicle; *MI*, metaphase I; *MII*, metaphase II; *PN*, pronuclei; *ET*, embryo transfer. ()—Figures in the brackets represented the number of oocytes and embryos. Bold terms highlighted pregnancy outcomes

Case report

Patient history

A 28-year-old Chinese woman and her 35-year-old Chinese husband with 2-year secondary infertility were seen at the Department of Reproductive Medicine, the Affiliated Hospital of Southwest Medical University. The woman had regular menstrual cycles, but her husband had severe asthenozoospermia confirmed on three occasions. Ten antral follicles in each ovary were seen at basal vaginal ultrasound scan in

October 2020. Blood samples of the patient revealed basal hormone concentrations on day 2 of the menstrual cycle, with a slightly high level of prolactin (PRL) (29.42 ng/ml). Normal levels of antimullerian hormone (AMH) (3.67 ng/ml), serum luteinizing hormone (LH) (5.17 mIU/ml), follicle-stimulating hormone (FSH) (8.78 mIU/ml), testosterone (TS) (76.17 ng/dl), progesterone (P) (0.21 g/ml), and estradiol (E2) (43.46 pg/ml) were revealed.

Ovarian stimulation, ICSI, and cryopreservation

The couple was counseled for ICSI for infertility due to severe asthenozoospermia. Ovarian stimulation was achieved using a gonadotropin-releasing hormone (GnRH) antagonist protocol with recombinant human follicle-stimulating hormone (150 IU, Merck Serono, Germany). After 12 days of ovarian stimulation, the patient's peak estradiol level was 3636.83 pg/ml, her luteinizing hormone level was 1.64 mIU/ml, and her progesterone level was 0.43 ng/ml. Ovulation was triggered for final oocyte maturation with recombinant human chorionic gonadotrophin (HCG) (250 µg, Merck Serono, Germany), and oocyte retrieval was performed at 36.5 h after HCG injection.

Ten oocytes were mature including a conjoined oocyte. The oocytes appeared equal in size, with one appearing as a metaphase II (MII) oocyte and the other one as an immature oocyte with a large vacuole. The site of fusion in the zonal region was morphologically remarkable because the zona pellucida (ZP) was discontinuous with a clear breach and an enlarged area (Fig. 1). The semen sample used for ICSI showed a sperm concentration of 25 million/ml, 25% motility with 0% forward progression, and 99.5% abnormal forms. ICSI was performed to all mature oocytes. At fertilization check 18 h post-ICSI, seven MII oocytes presented normal fertilization (two pronuclei, 2PN), including the MII of the conjoined oocyte. These embryos were cultured in sequential media (COOK, Australia). The fertilized conjoined oocyte cleaved to a high-quality 8-cell embryo on day 3 (Fig. 2a), and it reached a high-quality expanded blastocyst which was graded as 4BB according to the classification of Gardner and Schoolcraft [23] and frozen on day 5 (Fig. 2b). Another two high-quality cleavage-stage embryos resulted from morphologically normal mature oocytes were frozen on day 3. The remaining embryos reached two low-quality blastocysts frozen on day 6.

Thawing, embryo culture, and embryo transfer

Thawing of the two cryopreserved cleavage-stage embryos in January 2021 followed the thawing protocol (Thawing Media, Kitazato, Japan), and both were cultured until day 5. To help herniation, the ZP of each thawed cleavage-stage embryo was thinned, and a 20-mm hole on the ZP was opened using laser on day 3. On day 5, one reached the viable hatching blastocyst stage graded as 5BC (Fig. 2c), while the other embryo was blocked at cleavage stage. On the day of embryo transfer, the couple was informed with all the details about the blastocyst developed from the conjoined oocyte. However, they still requested to thaw and transfer the blastocyst with the other one graded as 5BC (Fig. 2c, d). The ZP of the blastocyst developed from conjoined oocyte was thinned using laser, but the degenerated immature oocyte was not removed from the complex prior to transfer (Fig. 2d). At time of transfer, the endometrial thickness measured 9.3 mm. Embryo transfer was successfully performed at the appropriate point in a hormone replacement cycle.

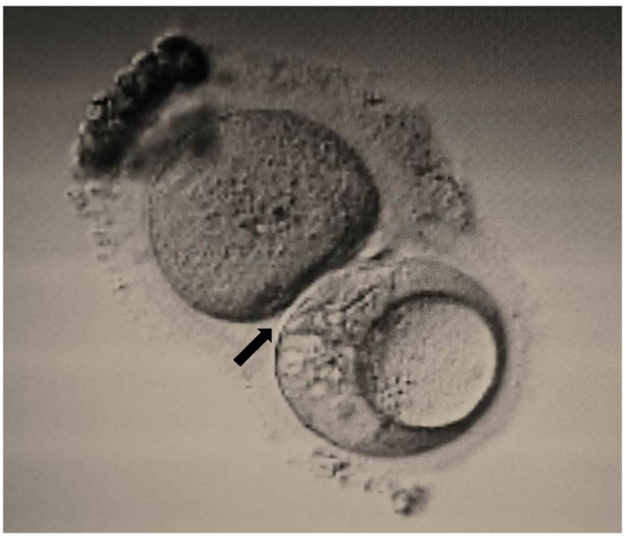

Fig. 1 A pair of conjoined oocytes arise from binovular follicles, which include a mature oocyte (MII) and an immature oocyte containing a large vacuole on day 0. The connected region of the zona pellucida is indicated by black arrow

The β-hCG level was positive 14 days after embryo transfer (1788.69mIU/ml). Successful pregnancy with two gestational sacs and fetal heartbeats was achieved 28 days after embryo transfer. The patient was routinely told to abstain from sexual intercourse in the hormone replacement cycle, which excluded the possibility of concomitant spontaneous pregnancy. Dizygotic twin girls, weighing 1600 g and 1700 g respectively, were delivered via cesarean section at 31 weeks' gestation, with no apparent abnormalities. Their Apgar scores at 1 and 5 min were 9–9 for both twins. The two infants, weighing 2200 g and 2400 g respectively, were discharged after 20 days of hospital observation in the neonatal department when their health indicators were normal. In addition, we followed up 100 days after birth, and the two babies had grown up healthy, weighing 5000 g and 5400 g respectively.

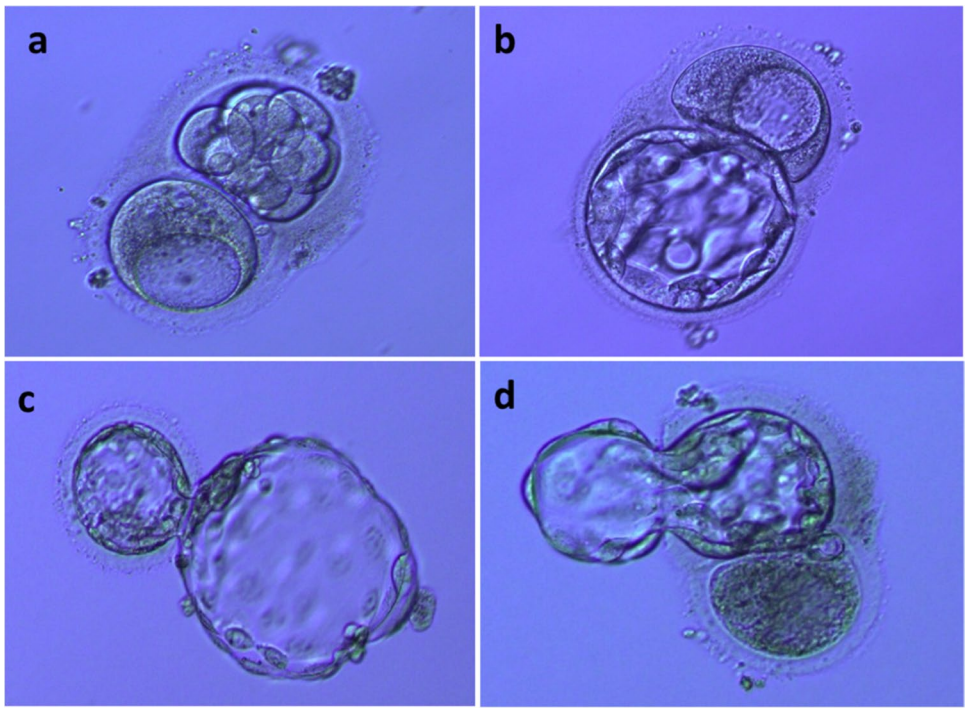

Fig. 2 The high-quality embryo from a conjoined oocyte and the hatching blastocyst from a separated oocyte. **a** A high-quality 8-cell embryo derived from the conjoined oocyte on day 3 after ICSI. **b** A high-quality expanded blastocyst (4BB) derived from the conjoined oocyte on day 5. **c** The hatching blastocyst (5BC) derived from a separated oocyte. **d** The hatching blastocyst derived from the conjoined oocyte and the degenerated immature oocyte still attached

Discussion and literature review

To our knowledge, this is the first reported live birth from a blastocyst derived from a conjoined oocyte after ICSI in a frozen embryo transfer cycle. Moreover, this study reports the first time that live birth of a conjoined oocyte is achieved without removing the degenerated immature oocyte. In addition, we reviewed the literature to evaluate the origin, incidence, safety, and significance of conjoined oocytes in reproductive health.

Origin and incidence of binovular follicles

Polyovular follicles have been found in histological investigation of ovaries, including animals and human [24]. Gougeon et al. [2] studied the results of 117 ovarian biopsies of 18- to 52-year-old women and reported that polyovular follicles and polynuclear oocytes were found in 98% of these adult women, and the vast majority (97.1%) of these structures was binovular. It was reported that the relative frequency of polyovular follicles was not age-dependent and varied between 0.06 and 2.44%. Conversely, Dandekar et al. [3] published the results of 251 laparoscopic egg retrievals and reported that 24% of oocyte-containing follicles were polyovular. However, it cannot be excluded that some of these oocytes originated from different follicles. Therefore, the present report concentrates on conjoined oocytes as the most reliable indication for true binovularity. The most probable and accepted mechanism for the formation of conjoined oocytes is that two individual germ cells fail to be separated by granulosa cells in early folliculogenesis [19]. Consequently, the pattern of ZP fusion may depend on the previous distance between the two germ cells [7]. Growth and zona formation of each oocyte would then lead to various patterns of zonal fusion, such as two oocytes sharing a common and intact single-layer ZP, or two oocytes each with an individual ZP but joined in a defined region, or two oocytes having an extraordinarily thin or even missing zona but also a modified, discontinuous structure (Table 1). Live birth has been reported in these three zonal fusion patterns of conjoined oocytes. It demonstrates that various patterns of zonal fusion have no adverse effect on fertilization, embryo development, and pregnancy outcome.

Safety of using conjoined oocytes

It is now well documented that binovular follicles represent anatural polymorphism rather than a pathological phenomenon [4, 25]. The probability that binovular follicles will ovulate is very low because of the low incidence rate [2, 5]. However, some reports in the literature concluded that the induction of multiple follicular growth by the gonadotropins would have been expected to increase the incidence of binovular follicles in programs of human assisted reproduction treatment [21, 26], although there is lack of evidence to show the underlying mechanism [2]. It is conceivable that the phenomenon of conjoined oocytes has been encountered in many laboratories. However, it is widely debated

whether these conjoined oocytes retrieved during the ART treatments should be used for attempts at fertilization, subsequent embryo development, and embryo transfer. It has been suggested that conjoined oocytes may be linked to dizygotic twins, chimerism [11], mosaicism, tetraploidy [24], and teratomas [27], but these data are limited, and results are inconclusive. Recently, the first occurrence of healthy dizygotic twin delivery was reported by Yasmin Magdi after a pair of conjoined blastocyst transfer [10]. It showed evidence to support the idea that a pair of mature conjoined oocytes may be a cause of dizygotic twinning. The appearance of tetraploidy, chimerism, and teratomas would require fertilization of two conjoined oocytes, an exchange of cells, or fusion of two developing embryos. However, clear evidence to support this idea is lacking. A group culture of hatching blastocysts might result in the joining of 2 separate embryos [28]. However, it has been reported to occur at a rate of approximately 1:1500 embryos [29]. Moreover, in instances with merely attached zonal regions, a preceding local dissolution of the zona has to be postulated [30]. Therefore, we surmised that the probability that fusion of two embryos developed from the conjoined oocytes will occur is also very low. Furthermore, in review of Table 1, conjoined oocytes are usually asynchronously mature, and one of the conjoined oocytes is immature. It appears that the immature oocyte generally demonstrates developmental arrest whereas the mature oocyte may be fertilized [5, 12]. Safran et al. [18] analyzed a pair of conjoined oocytes by fluorescence in situ hybridization (FISH) and concluded that each oocyte of conjoined oocytes represents an individual gamete with a chromosomal composition corresponding to its maturation stage. The sperm penetration and subsequent fertilization of primary oocytes are an extremely unusual phenomenon in mammals and only appear to occur on a regular basis in LT/Sv strain mice [31]. In three mammalian species, namely the horse [32], the fox [33], and the dog [34], sperm penetration does not in fact occur until after the oocyte has matured to metaphase of the second meiotic division. Studies of rabbit [35] showed that oocytes must occupy a certain position inside the follicle and reach the size that allows resumption of meiosis. Braden et al. [36] reported that oocyte penetrated by sperm prior to anaphase of the first meiotic division was not normally competent to initiate a proper "zona reaction." Nevertheless, the term "mosaicism" cannot be applied here, because two different spermatozoa are involved and because mosaicism, by definition, develops from only one zygote [30]. Rosenbusch [30] reviewed the literature and concluded that the currently available data did not support a role of conjoined oocytes in producing tetraploidy, chimeras, or mosaicism. In addition, Gougeon [2] suggested that the origin of benign cystic teratomas is not related to the presence of binovular follicles, as binovular follicles are found at all ages, whereas benign cystic teratomas are characteristic of young women. These reports imply that binovular follicles may play a role in producing dizygotic twins, but conjoined oocytes are not responsible for the majority of genetic abnormalities.

Significance of conjoined oocytes

We reviewed the literature to evaluate the significance of conjoined oocytes. A pair of conjoined oocytes were analyzed by FISH to conclude that the mature oocyte of conjoined oocytes was a normal, haploid MII oocyte before fertilization and could lead to the development of a chromosomally balanced embryo [18]. In review of Table 1, the published and current cases have shown that a total of 39 MII oocytes from the conjoined oocytes were attempted for fertilization, and 27 of them were normally fertilized (2PN). Twenty-five 2PN zygotes developed into embryos, including 11 blastocysts and 14 cleavage-stage embryos. However, there is low chance for embryo transfer derived from a conjoined oocyte due to having the other normally developing embryos. Fortunately, four case reports showed that five blastocysts derived from conjoined oocytes were successfully implanted and obtained live birth. Two of them reported live birth from conjoined oocytes, fertilized using ICSI in the fresh blastocyst transfer cycle [10, 15]. Another case resulting in live birth was reported by standard IVF from frozen-thawed blastocyst transfer [16]. In the present study, we report for the first time live birth of a blastocyst derived from a conjoined oocyte after ICSI in a frozen embryo transfer cycle. Unfortunately, another four case reports described that none of these cleavage-stage embryos derived from conjoined oocytes was successfully implanted [5, 7, 11, 12]. Jiao et al. [21] analyzed the characteristics of 18 patients with conjoined oocytes and reported that there was no significant difference in the rate of cleavage and D3 high-quality embryo between normal oocytes and conjoined oocytes, but the rate of blastocyst formation of conjoined oocytes decreased significantly. This indicates that blastocyst culture can further optimize selection of the cleavage-stage embryos developed from the conjoined oocytes. It is concluded that mature oocytes contained in conjoined-oocyte complexes can be fertilized by standard IVF or ICSI and have subsequent embryo development potential, which are recommended for blastocyst culture. The other normally developed embryos should be transplanted first, while the high-quality embryos developed from the conjoined oocytes should be kept frozen for future attempt. When patients have no high-quality embryos developed from oocytes with normal

morphology, the high-quality blastocysts resulted from the conjoined oocytes may be attempted to transfer with informed consent in a fresh or frozen embryo transfer cycle.

Previous cases have revealed asynchronous maturation of conjoined oocytes (Table 1). It is widely concerning how to deal with the immature oocyte of conjoined oocytes prior to embryo transfer. The conjoined oocyte might impair the ability of the embryo to expand and/or hatch from the zona due to their fusion in this region. Two previous reports recommended separation of the immature oocyte using diode laser from the complex prior to transfer and obtained live birth [15, 16]. Cummins et al. [15] reported that the immature oocyte of conjoined oocytes was removed via laser on day 3. Yano et al. [16] reported that a diode laser was used to thin the ZP prior to blastocyst transfer after removal of the degenerated unfertilized oocyte of a conjoined-oocyte complex. This study reports the first time that live birth of a conjoined oocyte is also achieved without removing the degenerated immature oocyte, indicating that the degenerated immature oocyte has no adverse effect on subsequent embryo development and pregnancy outcome. Nevertheless, zona pellucida hardening may be induced by cryopreservation and result in embryonic hatching difficulties [37]. Laser-assisted hatching (LAH) may overcome this problem [38]. LAH is performed at the appropriate point on frozen embryos, and we recommend retention of the degenerated immature oocyte from the complex prior to transfer when it is close to the inner cell mass of blastocyst.

Conclusions

In light of results from published and current cases, we further confirm previous reports that demonstrate that a mature oocyte from conjoined-oocyte complexes can be fertilized by standard IVF or ICSI and lead to the development of a blastocyst, subsequent pregnancy, and live birth. We reviewed the literature and concluded that blastocyst culture could further optimize selection of the cleavage-stage embryos developed from the conjoined oocytes and be beneficial to pregnancy outcome. The other normally developed embryos should be transplanted first, while the embryos developed from the conjoined oocytes should be kept frozen for future attempt. When patients have no high-quality embryos developed from oocytes with normal morphology, the high-quality blastocysts resulted from the conjoined oocytes may be attempted to transfer with informed consent. To our knowledge, this is the first case of live birth from a blastocyst derived from a conjoined oocyte fertilized using ICSI in a frozen embryo transfer cycle.

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Data availability All data generated or analyzed during this study are included in this published article.

Code availability Not applicable.

Declarations

Ethics approval All procedures followed were in accordance with the ethical guidelines of the Helsinki Declaration and were approved by the Human Ethics Committee of the Affiliated Hospital of Southwest Medical University.

Consent to participate As a case report, this was reported with the patient's informed consent.

Consent for publication Written informed consent for publication was obtained from all participants.

Conflict of interest The authors declare no competing interests.

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