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CASE REPORTS

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Conjoined human oocytes observed during assisted reproduction: description of three cases and review of the literature

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Abstract

Conjoined oocytes derived from binovular follicles have been suspected to play a role in producing dizygotic twins, mosaicism, tetraploidy or chimeras. This issue is discussed by presenting three examples for conjoined oocytes observed in our programme of human assisted reproduction and by including a review of corresponding cases. Our material comprises two associated immature oocytes and two cases in which an immature oocyte was attached to a mature oocyte. One of the mature oocytes was fertilized and transferred after cleavage without resulting in pregnancy. In the literature, another fifteen descriptions of conjoined oocytes were found. Here, simultaneous fertilization of both gametes occurred only once and there was no evidence for pregnancies arising from binovular follicles. These data suggest that binovular follicles do not cause dizygotic twins or genetic abnormalities.

Keywords: conjoined oocytes, binovular follicles, binovular zona pellucida, genetic abnormalities.

Introduction

Two conjoined oocytes within one follicle have occasionally been observed in programmes of human assisted reproduction [1–9], thus providing evidence for the assumption that some of the polyovular follicles known from histological studies of the ovary [10–12] may persist until ovulation. An investigation based on laparoscopic oocyte retrieval from stimulated cycles even concluded that 8% of oocyte-containing follicles were polyovular [13]. Here, however, the possibility that more than one follicle had been aspirated cannot be excluded with certainty. Therefore, the most reliable criteria for true binovularity are inclusion of two oocytes within a common zona pellucida or their fusion in the zonal region.

It has been suggested that binovular follicles may give rise to dizygotic twins and chimeras [1, 4], diploid-triploid mosaics [3], or tetraploidy [14]. Essential prerequisites for these events are the maturity, fertilizability and further developmental capacity of both oocytes. The present report describes three cases of conjoined female gametes and includes a review of the literature to evaluate whether binovular follicles could indeed play a role in the above-mentioned phenomena.

Patients, Methods and Results

Case No. 1

A 30-year-old nulligravida with regular menstrual cycles and no evidence for tubal or other factors of infertility was admitted to our programme of assisted reproduction because of her husband's severe oligo-asthenoteratozoospermia. Both partners had normal somatic karyotypes. Ovarian stimulation was performed with recombinant follicle stimulating hormone (FSH) and seven oocyte-cumulus complexes were retrieved by transvaginal, ultrasound-guided needle aspiration 36 hours after the administration of human chorionic gonadotropin (hCG). The first oocyte-cumulus complex contained a single immature oocyte in the germinal vesicle (GV) stage whereas the second revealed two conjoined GV-stage oocytes that were slightly different in size. The site of fusion was morphologically remarkable because the zona pellucida was discontinuous with a clear breach and an enlarged area (Figure 1A). This irregular thickening resembled a phenomenon denoted as "bilayering" by Veeck LL [7]. The conjoined oocytes degenerated during further in vitro culture without reaching metaphase I or II. Finally, five mature oocytes at metaphase II (MII) were available for intracytoplasmic sperm injection (ICSI) and three of them developed two pronuclei (PN). One of the pronuclear stages was cryo-preserved whereas the remaining two were allowed to cleave and transferred. However, the patient did not become pregnant during this cycle.

Case No. 2

A 30-year-old nulligravida with irregular menstrual cycles (26–35 days) and tubal risk factor was down-regulated with a gonadotropin releasing hormone (GnRH) agonist. Follicular stimulation was induced with recombinant FSH. The chromosomally normal couple opted for conventional *in vitro* fertilization (IVF) though distinct asthenozoospermia was ascertained in the husband's semen sample. Fourteen oocyte-cumulus complexes were obtained and inseminated but cumulus cell removal and assessment of pronuclear formation on the following morning revealed complete fertilization

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failure. All gametes proved to be mature but one of them was attached to a smaller GV-stage oocyte. The two cells were separated by a thin but seemingly intact zona pellucida (Figure 1B).

Case No. 3

A 32-year-old nulligravida with regular menstrual cycles and tubal occlusion was down-regulated with a GnRH agonist and received ovarian stimulation with human menopausal gonadotropin (hMG). ICSI was scheduled for the first trial of the chromosomally normal couple because the husband's semen analysis showed teratozoospermia. Of 19 oocytes retrieved, two were immature at GV stage and 17 were available for ICSI. One of these mature gametes was associated with an immature GV-stage oocyte. Both cells appeared to have an individual zona pellucida. Following sperm injection, the MII oocyte developed two PN and cleaved to a two-cell embryo whereas the immature oocyte did not undergo further maturation (Figure 1, C–E). In total, regular formation of two PN occurred in 12 of the injected oocytes, one was abnormally fertilized (3PN) and four were not fertilized. The embryo with the attached GV-stage oocyte was transferred together with a four-cell embryo. Pregnancy was not achieved but occurred in one of the following cycles after transfer of embryos obtained from frozen-thawed 2PN-stages.

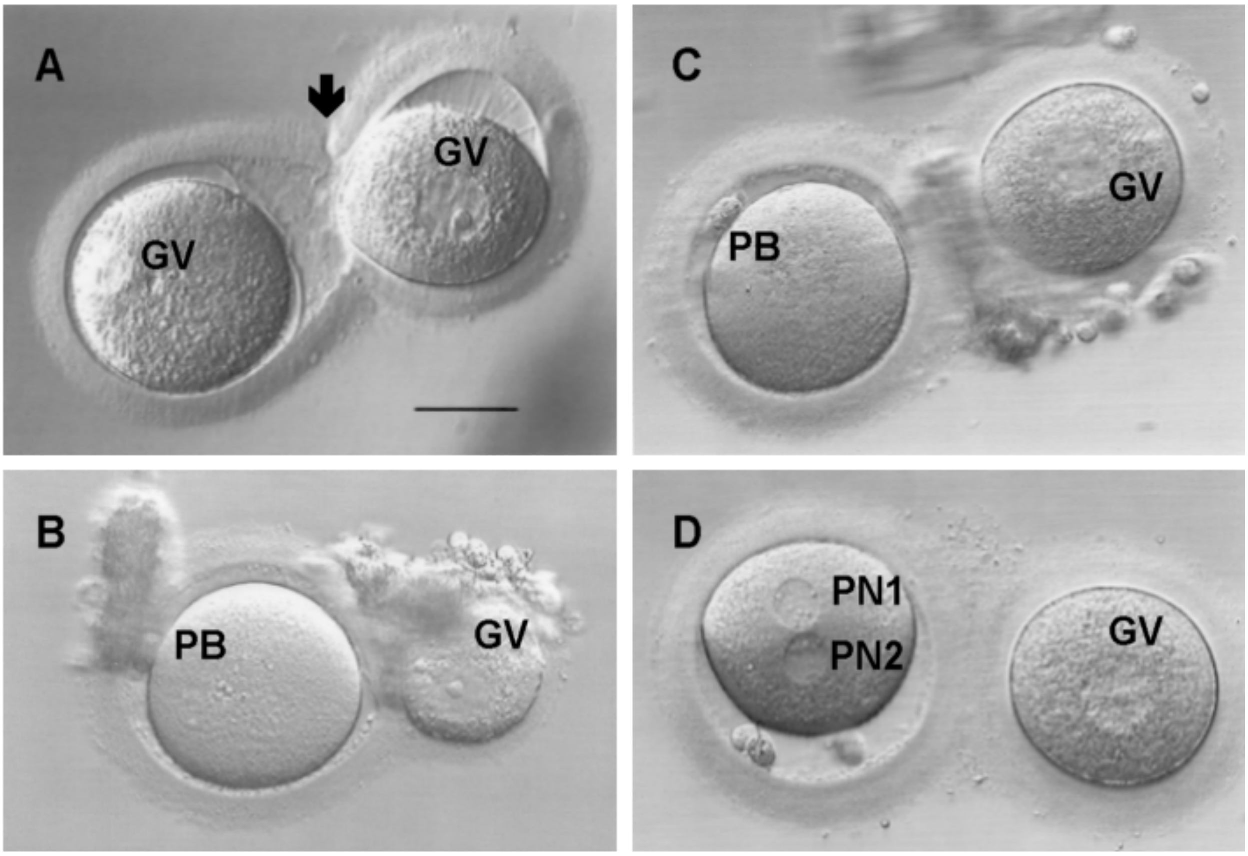

Figure 1 – ***(A) Case No. 1: Two conjoined immature oocytes, both displaying a germinal vesicle (GV). The zona pellucida at the site of fusion has a modified, discontinuous structure and a breach (arrow). (B) Case No. 2: A mature MII oocyte attached to a smaller GV-stage oocyte. The first polar body (PB) and the connecting thin zonal region are partly obscured by cumulus cells that could not be removed completely. (C–E) Case No. 3: (C) Two associated oocytes in the MII and GV-stage, respectively. Both gametes appear to have their individual zona pellucida; (D) After ICSI, the mature oocyte developed two pronuclei (PN1 and PN2); (E) The bipronuclear oocyte has cleaved to a two-cell embryo whereas the connected cell remained in the GV-stage. Scale bar = 50 µm.***

Discussion

Multiovular follicles have been found in ovarian biopsies of 18- to 52-year-old women and the vast majority (97.1%) of these structures was binovular, i.e. they contained two oocytes [12]. The most probable explanation for the formation of binovular follicles is that two individual germ cells failed to be separated by granulosa cells during the early stage of folliculogenesis [8]. Consequently, the pattern of zonal fusion may depend on the previous distance between the two germ cells. Growth and zona formation of each oocyte would then lead either to conjoined gametes that share a common and intact zona or to two oocytes with an individual zona that are connected in a defined region. Further alternatives (Table 1) are an extraordinarily thin or even missing zona but also a modified, discontinuous structure as shown in Figure 1A. Of course, it is conceivable that a binovular follicle contains two oocytes surrounded by their individual cumulus cells without fusion in the zonal region. In fact, Ron-El R *et al.* [5] observed nine follicular aspirates containing two adjacent oocyte-cumulus complexes that could easily be separated whereas in another case, two oocytes with individual corona radiata were found within a single cumulus complex. Dandekar PV *et al.* [13] even reported that 61 out of 251 laparoscopies (24%) yielded poly-ovular follicles, including five cases with three or four oocytes. However, it cannot be excluded that some of these oocytes originated from different follicles and therefore, the present report concentrates on conjoined oocytes as the most reliable indication for true binovularity.

Table 1 – ***Reported cases of conjoined oocytes***

Zona structure at junction	Maturity of gametes		Fertilization		Further development	References
	Oocyte 1	*Oocyte 2*	*Oocyte 1*	*Oocyte 2*		
Oocytes with own, connected zona	MII [a]	MII [a]	2PN	2PN	Cleavage and ET of one embryo after mechanical separation, no pregnancy	Zeilmaker GH *et al.* [1]
Oocytes share an intact single layer	?	MII [a]	--	2PN	Cleavage and ET, no pregnancy	Ben-Rafael Z *et al.* [2]
Oocytes share an intact single layer	GV	MII	--	--	Fixed for analysis	Fishel S *et al.* [3]
Oocytes share an intact single layer	GV	MII [a]	--	2PN	Frozen for analysis at blastocyst stage	Hartshorne GM *et al.* [4]
Oocytes share an intact single layer	GV	GV	--	--	--	Ron-El R *et al.* [5]
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	MII	--	2PN	Cleavage, no ET	Safran A *et al.* [6]
Oocytes share an intact single layer	GV	MII	--	2PN	Fixed for analysis after cleavage	
Oocytes share an intact single layer	GV	MI	?	?	?	Veeck LL [7]
Oocytes share an intact single layer	GV	MII	--	2PN	Cleavage to blastocyst, no ET	Vicdan K *et al.* [8]
Oocytes with own, connected zona	GV	MII	?	?	?	Kousehlar M *et al.* [9]
Thin or not present	GV	?	?	?	?	
Modified structure (see text)	GV	GV	--	--	--	Present report
Oocytes share an intact single layer	GV	MII	--	--	--	
Oocytes with own, connected zona	GV	MII	--	2PN	Cleavage and ET, no pregnancy	

[a] Maturity deduced from the fact that the oocyte was normally fertilized (formation of 2PN). ? – Not indicated; GV – Germinal vesicle; MII – Metaphase II; PN – Pronuclei; ET – Embryo transfer.

Each of the two conjoined oocytes represents an individual female gamete with a chromosomal constitution that corresponds to its stage of maturity. Safran A *et al.* [6] who analyzed a pair of conjoined oocytes by fluorescence *in situ* hybridization (FISH) have provided evidence for this assumption. They reported that the results were consistent with a diploid germinal vesicle in the immature cell whereas the conjoined embryo that developed after ICSI had a diploid female chromosome constitution. This allowed concluding that the larger cell was a normal, haploid MII oocyte before fertilization.

It has repeatedly been suggested that conjoined oocytes may play a role in producing dizygotic twins or genetic abnormalities [1, 3, 4, 14]. For instance, one group of investigators surmised that the close contact of two embryos might lead to the formation of a single conceptus after dissolution of the zona pellucida prior to implantation. Alternatively, a dizygotic twin could have developed that, following an exchange of cells, would represent "the best known type of chimerism" [1]. Moreover, fusion of two closely associated, fertilized oocytes has been discussed as a possible origin of tetra-ploidy [14] whereas Fishel S *et al.* [3] reflected on the formation of a diploid-triploid mosaic individual. This event would require fusion of a diploid embryo, arising from monospermic fertilization of a MII oocyte, and a digynic triploid embryo, resulting from monospermic fertilization of a conjoined primary oocyte. Of note, Fishel S *et al.* [3] expected a mosaic rather than chimeric individual because in their opinion, the conjoined oocytes were probably derived from the division of a single egg.

These considerations show that the actual appearance of genetic abnormalities would either require normal fertilization of two mature female gametes or concomitant fertilization of a primary and secondary oocyte. However, clear evidence to support this idea is lacking. Fertilization of both oocytes occurred only once whereas in the other cases, one of the gametes was immature and did not display developmental potential. In about 50% of the reported cases, one of the conjoined oocytes was fertilized (Table 1). This indicates an asynchronous maturation of conjoined gametes or generally, oocytes from multiovular follicles. Studies in the rabbit [15] suggested that an oocyte must occupy a certain position inside the follicle and reach a size that allows resumption of meiosis. Therefore, oocytes that are not in the correct position will hardly undergo maturation. However, polyovular follicles were found in the ovaries of alligators after exposure to environmental contaminants [16]. It would be highly interesting to know how such exogenous substances influence the formation and maturation of polyovular follicles in the human.

Conclusions

The currently available data from programs of assisted reproduction do not argue for a crucial role of binovular follicles in the formation of dizygotic twins, mosaicism, tetraploidy or chimeras. Any discussion of this topic, however, must take into account that only few reports on conjoined oocytes have been published most of which are older than a decade. It is conceivable that the phenomenon of binovular follicles has been encountered in many laboratories and was considered to be without any significance. Additional information on the developmental potential of conjoined gametes and oocytes enclosed within a common cumulus mass is definitely required. In particular, the impact of chemicals with estrogenic activity on human folliculogenesis deserves increased attention.

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