Case Reports

Evidence using a shared oocyte pool that the sperm rather than the oocyte in some cases may be responsible for the production of embryos with a high percentage of fragmented blastomeres – Case report

J.H. Check, *M.D., Ph.D.*; **H. Cochrane,** *B.A.*; **W. Yuan,** *Ph.D.*; **C. Wilson,** *B.A.*

The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, NJ (USA)

Summary

Purpose: Hypothesis – a sperm defect rather than an oocyte problem may occasionally be responsible for blastomere fragmentation.

Methods: The morphology of embryos in pairs of women producing embryos from a common pool of oocytes but fertilized by two different sperm sources was performed to see if a pair could be detected where one woman produced embryos with very little blastomere fragmentation vs the other woman having embryos with extensive fragmentation.

Results: An oocyte donor was identified who produced 49 oocytes resulting in 17 embryos for the infertile donor to evaluate and 13 for the recipient. Almost all of the donor embryos showed extensive fragmentation vs very little for the recipient embryos.

Conclusion: The sperm, in some cases at least, may be the etiologic factor for blastomere fragmentation.

Key words: Blastomere; Fragmentation; Shared oocytes; Sperm.

Introduction

Both apopotosis and necrotic processes have been suggested as causes of blastomere fragmentation in human embryos [1]. However, a definitive etiology has not been determined.

Sperm cells bear specific antigen moieties that are incorporated into the zygote at the time of fertilization. Sperm surface antigens remain on the surface of the zygote during the first divisions and are subsequently internalized and/or degraded [2]. In some cases, e.g., in the mouse embryo, the paternal genome can have very deleterious interactions with the maternal cytoplasm, inducing destruction of the embryos before blastocyst formation [3].

There have been some data in humans suggesting that poor sperm quality can be associated with poor embryonic development [4, 5]. Some women consistently produce embryos with a high degree of fragmentation following several in vitro fertilization (IVF) attempts. Evidence will be provided that the problem of recurrent production of highly fragmented embryos may be related to a sperm factor rather than an egg factor or other influences.

Case Report

The comparison of outcome of a single oocyte pool fertilized by two different sperm, i.e., shared oocytes used as a source of donor oocytes, may provide unique insights as to etiology of infertility [6]. One such couple is described thus suggesting that the sperm, in some cases, may be responsible for blastomere fragmentation.

The couple with the high percentage of fragmented embryos had the female partner who was the egg donor. She was 35 years old with a 4-year history of primary infertility. The cause of the infertility was ascribed to a male factor, in that despite a normal sperm concentration of 118×10^6/ml and motility of 42.3% with 14.8% sperm with rapid linear motion and 8% normal morphology using strict criteria, there was a significant problem with antisperm antibodies (82% IgG and 77% IgA) using the direct immunobead assay. Furthermore, there was a low hypoosmotic swelling test score of 47% [7, 8].

The donor had 49 oocytes retrieved following controlled ovarian hyperstimulation with the luteal phase leuprolide acetate-gonadotropin regimen (beginning at 300 IU a day of recombinant follicle stimulating hormone (FSH) (Follistim®, Organon, Inc.) decreasing to 225 IU and eventually 150 IU recombinant FSH). The donor received 24 oocytes. Following intracytoplasmic sperm injection (ICSI), 17 of the 24 oocytes (70.8%) fertilized. All embryos were cryopreserved at the 2 pronuclear stage because of the risk of ovarian hyperstimulation syndrome. All 17 of the donor's embryos were allowed to cleave and 13 were transferred over three cycles and four were discarded because of cleavage arrest. One hundred percent of the donor's embryos had > 25% fragmentation (Table 1) and four (30.7%) had more than 50% fragmentation. She failed to conceive.

The recipient received 25 oocytes which were fertilized conventionally and 13 (52%) fertilized. Three embryos were transferred fresh and two were discarded because of cleavage arrest. Thirty-three percent had > 25% fragmentation (Table 1) but none had > 50%. She conceived and delivered.

Revised manuscript accepted for publication November 3, 2003

The remaining eight frozen embryos were donated to another woman. Subsequently all embryos were thawed and 12.5% had > 25% fragmentation (Table 1) but none had > 50%. She failed to conceive after the transfer of three embryos and three were refrozen. Two embryos were discarded because they failed to cleave. As of date, the three remaining embryos are still frozen.

Table 1. — *Blastomere fragmentation according to source of sperm.*

	Number of cleaved fresh embryos	Number of fresh embryos with > 25% fragmentation	Number of frozen embryos cleaved	Number of frozen embryos with > 25% fragmentation
Donor	0	0	13	13
Recipient	3	1	8	1

Discussion

In the human, antisperm antibodies may interfere considerably with pregnancy outcome, causing a developmental block just before genomic activation [9]. The negative effect could be related to antibodies to cleavage signal proteins or to the regulatory product of the gene immunoneutralization [9].

There have been some data suggesting that the process of ICSI itself could lead to an increased rate of fragmentation without causing decreased implantation [10]. However, other studies suggest that ICSI improved embryo quality [11]. The recipient's share of the oocytes were fertilized by conventional insemination.

Nagy *et al.* found a higher proportion of poor quality embryos following IVF with ICSI for sperm with autoantibodies, as compared to ICSI with antibody negative sperm [12]. However, an association with sperm autoantibodies and poor embryo morphology was not found in the study by Clarke *et al.* [13].

A subnormal hypoosmotic swelling test allows normal fertilization rates and cleavage to the multi-cell embryo stage, but implantation is markedly inhibited [8]. The defect appears to be related to the transfer of a toxic factor from the sperm to the zona pellucida [14]. The fact that ICSI with sperm with low hypoosmotic swelling test scores obviates the problem and leads to normal pregnancy rates, supports this hypothesis [15]. However, in a study of shared oocyte cycles, where one male partner had a hypo-osmotic swelling test score ≥ 50% and the other male partner had a score < 50%, there were no differences in embryo morphology [16].

The study presented here, comparing morphology of embryos after fertilization of a common pool of oocytes by two different sources of sperm clearly demonstrates that the sperm, at least in some cases, may be a contributing cause for embryo blastomere fragmentation. Conflicting data in the literature does not allow conclusions as to whether the presence of sperm autoantibodies or toxic factors leading to a functional defect of the sperm membrane (as manifested by low hypoosmotic swelling test scores) was responsible, or whether some unidentified factor independent of the aforementioned sperm factors was responsible.

We will continue to search for cases in our shared oocyte program where a high percentage of embryos have extensive fragmentation of blastomeres, so that by evaluating the other pairs we may ascertain whether the male factor is a frequent or rare associated factor when extensive blastomere fragmentation is present.

References

[1] Juriscova A., Varmuza S., Casper R.: "Programmed cell death and human embryo fragmentation". *Mol. Hum. Reprod.*, 1996, *2*, 93.
[2] O'Rand: "The presence of sperm specific isoantigens on the egg following fertilization". *J. Exp. Zool.*, 1977, *202*, 267.
[3] Renard J.P., Barra J., Babinet C.: "Action of the paternal genome at the beginning of embryonic development". *Reprod. Nutr. Dev.*, 1988, *28*, 1541.
[4] Ron-El R., Nachum H., Herman A., Golan A., Caspi E., Soffer Y.: "Delayed fertilization and poor embryonic development associated with impaired semen quality". *Fertil. Steril.*, 1991, *55*, 338.
[5] Janny L., Vye P., Pouly J.L., Hazout A., Dumont M., Nicollet B., Menezo Y.: "Cocultures: diagnostic and therapeutic value". *Contracept. Fertil. Sex*, 1993, *21*, 391.
[6] Check J.H.: "The shared donor oocyte program: the advantages and insights it provides in determining etiologic factors of infertility". *Clin. Exp. Obstet. Gynecol.*, 2002, *29*, 229.
[7] Check J.H., Epstein R., Nowroozi K., Shanis B.S., Wu C.H., Bollendorf A.: "The hypoosmotic swelling test as a useful adjunct to the semen analysis to predict fertility potential". *Fertil. Steril.*, 1989, *52*, 159.
[8] Check J.H., Stumpo L., Lurie D., Benfer K., Callan C.: "A comparative prospective study using matched samples to determine the influence of subnormal hypo-osmotic test scores of spermatozoa on subsequent fertilization and pregnancy rates following in-vitro fertilization". *Hum. Reprod.*, 1995, *10*, 1197.
[9] Naz R.R.: "Effect of antisperm antibodies on early cleavage of fertilized ova". *Biol. Reprod.*, 1992, *48*, 130.
[10] Frattarelli J.L., Leondires M.P., Miller B.T., Segars J.H.: "Intracytoplasmic sperm injection increases embryo fragmentation without affecting clinical outcome". *J. Assist. Reprod. Genet.*, 2000, *17*, 207.
[11] Yang D., Shahata M.A., al-Bader M., al-natsha S.D., al-Flamerzia M., al-Shawaf T.: "Intracytoplasmic sperm injection improving embryo quality: Comparison of the sibling oocytes of non-male-factor couples". *J. Assist. Reprod. Genet.*, 1996, *13*, 351.
[12] Nagy Z.P., Verheyen G., Liu J., Joris H., Janssenwillen C., Wisanto A. *et al.*: "Results of 55 intracytoplasmic sperm injection cycles in the treatment of male-immunological infertility". *Hum. Reprod.*, 1995, *10*, 1775.
[13] Clarke G.N., Bourne H., Baker H.W.G.: "Intracytoplasmic sperm injection for treating infertility associated with sperm autoimmunity". *Fertil. Steril.*, 1997, *68*, 112.
[14] Check J.H., Katsoff D., Check M.L.: "Some semen abnormalities may cause infertility by impairing implantation rather than fertilization". *Med. Hypoth.*, 2001, *56*, 653.
[15] Check J.H., Katsoff D., Check M.L., Choe J.K., Swenson K.: "In vitro fertilization with intracytoplasmic sperm injection is an effective therapy for male factor infertility related to subnormal hypo-osmotic swelling test scores". *J. Androl.*, 2001, *22*, 261.
[16] Katsoff D., Check M.L., Check J.H.: "Evidence that sperm with low hypoosmotic swelling scores cause embryo implantation defects". *Arch. Androl.*, 2000, *44*, 227.

Address reprint requests to:
J.H. CHECK, M.D., Ph.D.
7447 Old York Road
Melrose Park, PA 19027 (USA)

Prenatal diagnosis of pentalogy of Cantrell: A case report

L. Jochems, Y. Jacquemyn, B. Blaumeiser

Department of Obstetrics, Gynaecology and Genetics, Antwerp University Hospital UZA, Edegem (Belgium)

Summary

Pentalogy of Cantrell is a very rare congenital disorder characterized by ectopia cordis in combination with an abdominal wall defect. A case diagnosed prenatally at 25 weeks' gestational age is presented.

Key words: Ectopia cordis; Cantrell; Omphalocele; Prenatal diagnosis.

Introduction

Pentalogy of Cantrell consists of [1] a midline supraumbilical abdominal wall defect (most frequently resulting in omphalocele), [2] a defect of the lower sternum demonstrating as ectopia cordis, [3] diaphragmatic hernia due to a deficiency of the anterior diaphragm [4] a defect in the diaphragmatic pericardium and [5] intracardiac malformation. Less than 100 cases have been published. We report a case diagnosed prenatally by ultrasound.

Case Report

A 25-year-old primigravid women presented for routine ultrasound examination at 25 weeks' gestational age. Neither the patient nor her husband had a family history of congenital anomalies. On ultrasound a distal sternal defect with ectopia cordis (Figure 1) was seen in connection with an omphalocele, a ventricular septal defect and an overriding aorta. Amniocentesis was performed which showed a normal female karyotype and the amniotic fluid level of alfafetoprotein was within normal limits for the gestational age. The patient was counseled by the paediatric cardiologist and the neonatologist and the couple decided to terminate the pregnancy.

The neonate (Figure 2) clearly showed an omphalocele. At autopsy the median part of the diaphragm was absent as was part of the pericardium; tetralogy of Fallot, absence of the ductus arteriosus and a persistent left superior vena cava entering the left atrium were also noted. The final diagnosis matches complete pentalogy of Cantrell.

Discussion

Cantrell, Haller and Ravitch in 1958 described a syndrome consisting of a defect of the lower sternum, midline supraumbilical abdominal wall defect, deficiency of the anterior diaphragm, defect in the diaphragmatic pericardium and congenital intracardiac defects [1]. Few cases manifesting complete pentalogy have been reported since then and we were able to document only 25 cases of complete pentalogy of Cantrell diagnosed prenatally, including our own [2-15]. A prevalence of 5.5 per million live births has been suggested [16]. The syndrome is considered to be part of a spectrum of thoracoabdominal midline defects. Survival is rare and depends mainly on the cardiac malformation.

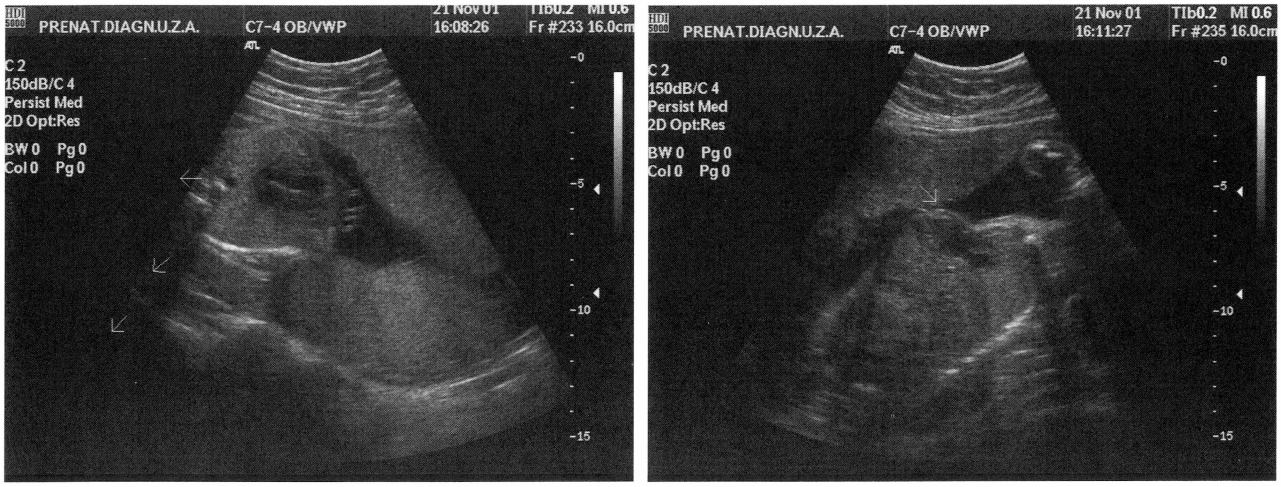

Figure 1. — Prenatal ultrasonographic image demonstrating ectopia cordis.

Revised manuscript accepted for publication February 13, 2004

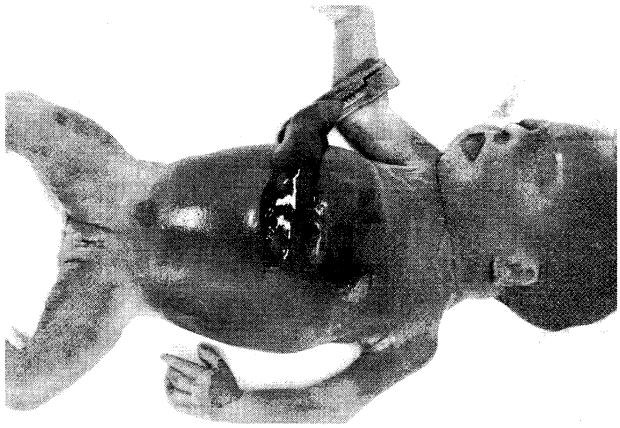

Figure 2. — Postmortem image showing ectopia cordis and omphalocele.

The etiology is uncertain and the genetics are unclear. Both concordance and discordance have been reported in twins; familial occurrence has been reported only once [17].

Antenatal ultrasonographic visualization of a ventral wall defect associated with ectopia cordis is highly suggestive of Cantrell's pentalogy and the earliest prenatal diagnosis described is at 11 weeks [8].

The parents should be informed of the poor prognosis in case of complete pentalogy of Cantrell (8% survival). The survival rate has been reported to be more optimistic in incomplete forms with survival rates up to 60%, especially those not demonstrating intracardiac defects [10, 18].

References

[1] Cantrell J.R., Haller J.A., Ravitch M.M.: "A syndrome of congenital defects involving the abdominal wall, sternum, diaphragm, pericardium and heart". *Surg. Gynecol. Obstet.*, 1958, *107*, 602.

[2] Harrison M.R., Filly R.A., Stanger P., de Lorimier A.A. : "Prenatal diagnosis and management of omphalocele and ectopia cordis". *J. Pediatr. Surg.*, 1982, *17*, 64.

[3] Mercer L.J., Petres R.E., Smeltzer J.S.: "Ultrasonic diagnosis of ectopia cordis". *Obstet. Gynecol.*, 1983, *61*, 523.

[4] Haynor D.R., Shuman W.P., Brewer D.K.: "Imaging of fetal ectopia cordis: roles of sonography and computerised tomography". *J. Ultrasound Med.*, 1984, *3*, 25.

[5] Todros T., Presbitero P., Montemuro D.: "Prenatal diagnosis of ectopia cordis". *J. Ultrasound Med.*, 1984, *3*, 429.

[6] Baker M.E., Rosenberg E.R., Trofatter K.F., Imber M.J., Bowie J.D.: "The in utero findings in twin pentalogy of Cantrell". *J. Ultrasound Med.*, 1984, *3*, 525.

[7] Ghidini A., Sirtori M., Romero R., Hobbins J.C.: "Prenatal diagnosis of pentalogy of Cantrell". *J. Ultrasound Med.*, 1988, *7*, 567.

[8] Bognoni V., Qurtuccio A., Quartuccio A.: "First-trimester sonographic diagnosis of Cantrell's pentalogy with exencephaly". *J. Clin. Ultrasound.*, 1999, *27*, 276.

[9] Schuppler U., Weisner D., Schollmeyer T., Grillo M., Franz W.: "Combination of Cantrell pentalogy and amniotic band syndrome: a case report". *Zentralbl. Gynakol.*, 1994, *116*, 115.

[10] Fernandez M.S., Lopez A., Vila J.J., Lluna J.: "Cantrell's pentalogy. Report of four cases and their management". *Pediatr. Surg. Int.*, 1997, *12*, 428.

[11] Hsieh Y.Y., Lee C.C., Chang C.C., Tsai H.D., Hsu T.Y., Hsu T.Y., Tsai C.H.: "Prenatal sonographic diagnosis of Cantrell's pentalogy with cystic hygroma in the first trimester". *J. Clin. Ultrasound.*, 1998, *26*, 409.

[12] Bonilla-Musoles F., Machado L.E., Bailao L.A., Osborne N.G., Raga F.: "Abdominal wall defects: two- versus three-dimensional ultrasonographic diagnosis". *J. Ultrasound Med.*, 2001, *20*, 379.

[13] Leon G., Chedraui P., San Miguel G.: "Prenatal diagnosis of Cantrell's pentalogy with conventional and three-dimensional sonography". *J. Matern. Fetal Neonatal. Med.*, 2002, *12*, 209.

[14] Pollio F., Sica C., Pacilio N., Maruotti G.M., Mazzarelli L.L., Cirillo P. *et al.*: "Pentalogy of Cantrell: first trimester diagnosis and association with multicystic dyplastic kidney". *Minerva Ginecol.*, 2003, *55*, 363.

[15] Nanda S., Nanda S., Agarwal U., Sen J., Sangwan K.: "Cantrell's syndrome - a report of two cases with one atypical variant". *Arch. Gynecol. Obstet.*, 2003, *268*, 331.

[16] Carmi R., Boughman J.A.: "Pentalogy of Cantrell and associated midline anomalies: a possible ventral midline developmental field". *Am. J. Med. Genet.*, 1992, *42*, 90.

[17] Martin R.A., Cuniff C., Erickson L.: "Pentalogy of cantrell and ectopia cordis, a familial development field complex". *Am. J. Med. Gen.*, 1992, *42*, 839.

[18] Vazquez-Jimenez J.F., Mueller E.G., Daebritz S.: "Cantrell's syndrome: a challenge to the surgeon". *Ann. Thorac. Surg.*, 1998, *65*, 1178.

Address reprint requests to:
Y. JACQUEMYN, M.D.
Department of Obstetrics
Gynaecology and Genetics
Antwerp University Hospital UZA
Wilrijkstraat 10
2650 Edegem (Belgium)