

· *Case Report* ·

# Clinical pregnancies and livebirths achieved by intracytoplasmic injection of round headed acrosomeless spermatozoa with and without oocyte activation in familial globozoospermia: case report

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## Abstract

We report the successful outcome of intracytoplasmic sperm injection (ICSI) treatment in two siblings with familial globozoospermia. After controlled ovarian hyperstimulation and oocyte pick-up, retrieved oocytes were mechanically activated before ICSI and a fertilization rate of 33.3% was achieved in the first case. The second couple underwent ICSI without oocyte activation and a 9.1% fertilization rate was obtained. The transfer of two grade I embryos in the first couple and one grade I embryo in the second couple resulted in clinical pregnancies with healthy livebirths. It was concluded that the main problem of cases with globozoospermia is a low fertilization rate, and even though ICSI and oocyte activation can increase this rate it is not necessarily needed to achieve a pregnancy. (*Asian J Androl* 2008 Mar; 10: 332–336)

**Keywords:** intracytoplasmic sperm injection; spermatozoa; acrosome; scanning electron microscopy

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## 1 Introduction

Globozoospermia is a severe form of terato-zoospermia characterized by round-headed acrosomeless spermatozoa [1]. Analysis of the incidence of cases with globozoospermia demonstrated a history of consanguinity and a familial occurrence. Familial cases of globozoospermia suggest that this pathology has genetic origins, but the mode of inheritance remains unexplained, proba-

bly showing X-linked, sex-restricted dominant, or autosomal recessive modes of inheritance [2]. Some genes responsible for globozoospermia in mice were identified [3].

Two types of globozoospermia have been described in previous studies. Type I is characterized by a complete lack of acrosome and acrosomal enzymes, whereas type II has some acrosomal covering with a conical nucleus, which may be surrounded by large droplets of cytoplasmic material indicating degenerative changes. Some reports described high levels of sperm aneuploidy and abnormalities of chromatin packaging in globozoospermia [4, 5]. In addition, the failure of spermatid differentiation causes abnormalities of the acrosome, nucleus, manchette, postacrosomal sheath, posterior ring and the ectoplasmic specializations surrounding the sper-

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matid [6]. It has been known that round-headed spermatozoa not only lack acrosomes but also have deformed tails [7] and disorganized mid-pieces [8].

The round-headed spermatozoa cannot penetrate the zona pellucida of an oocyte and therefore cannot provide a healthy fertilization, leading to male infertility. Therefore, globozoospermia was previously thought to be a sterilizing pathology of the human male. After recent advances in assisted reproductive technologies, particularly the intracytoplasmic sperm injection (ICSI), few successful fertilization or pregnancies have been reported [9–11]. However, even using ICSI treatment, the fertilization rates were still poor and this was attributed to the inability of the round-headed sperm to activate an oocyte mainly because of premature chromatin condensation [12]. There are also reports about the effects of centrosomal dysfunction of the round-headed spermatozoa on poor fertilization rates after ICSI treatment [13].

In this report, we presented the successful outcome of ICSI in two siblings with familial complete globozoospermia detected by light and electron microscopy.

## 2 Case report

Two brothers were admitted to our center after 15 and 8 years of primary infertility. The ages of males were 35 and 31 and their wives were 37 and 26 years old, respectively. Neither of the brothers reported any exposure to potentially toxic chemicals, radiation or other potential environmental xenobiotics. Both brothers had normal karyotypes (46, XY) in their peripheral blood samples. Their hormonal profiles were within normal range. A basic semen analysis revealed  $81.6 \times 10^6$  total sperm count with a 21% total and 5% progressive motility in the first case and  $210 \times 10^6$  total sperm count with a 36% total and 13% progressive motility in the second case. Strict morphological evaluation after SperMac (Conception Technologies, San Diego, CA, USA) staining method for human spermatozoa showed 100% round-headed spermatozoa in both cases (Figure 1).

Ultrastructural characteristics of the round-headed spermatozoa were evaluated by scanning electron microscope (JEOL JSM-6060LV; JEOL Technics Ltd., Akishima-Shi, Tokyo, Japan). Sperm samples were fixed in 2.5% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer, washed in phosphate buffer saline, dehydrated in alcohol gradients, prepared for microscopy after critical point drying, and evaluated at 15 kV acceleration. De-

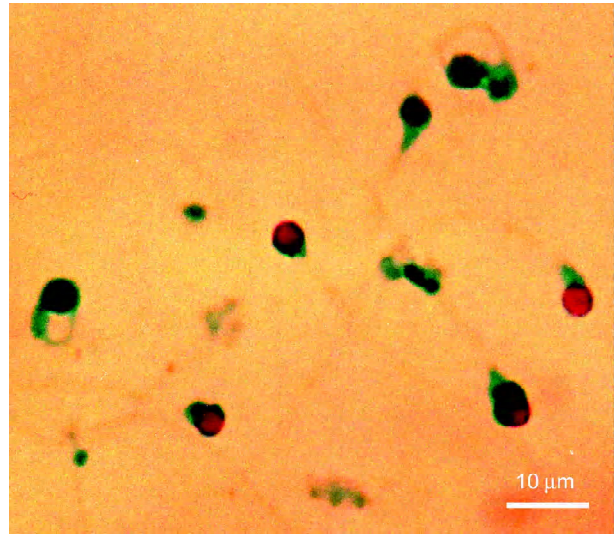


Figure 1. SperMac staining of round headed acrosomeless spermatozoa ( $\times 1\,000$ , Bright field). Note the spherical red nucleus of cells indicating the absence of acrosome.



Figure 2. Scanning electron micrograph of round headed acrosomeless spermatozoa. Spherical heads without acrosomes are shown. Severe neck, midpiece and tail defects are visible ( $\times 5\,000$ ).

tailed evaluation of the cells under SEM pointed out some severe midpiece and tail abnormalities (Figure 2).

After giving detailed information on outcome, both couples decided to undergo ICSI treatment. The gynecological and infertility work-up of the female partners revealed no abnormality. A microdose-flare protocol was preferred in the first case because of the age and basal

ultrasonographic evaluation of the ovaries and long protocol in the second case was carried out. In the first case, 450 IU recombinant FSH (rFSH)/day (Puregon; Organon, Istanbul, Turkey) s.c. and 80 mg leuprolide acetate/day (Lucrin; Abbot, Istanbul, Turkey) b.i.d. s.c. were applied. In the second case, 225 IU rFSH with 0.5 mg leuprolide acetate/day s.c. was used. Final oocyte maturation and ovulation stimulation was provided by 10 000 IU of HCG (Pregnyl; Organon, Istanbul, Turkey). Thirty-five hours later, oocytes were retrieved by transvaginal follicle aspiration under ultrasound guidance with a total intravenous anesthesia. Six and 11 metaphase II oocytes were obtained respectively. After denudation by hyaluronidase treatment, ICSI procedure was performed by using Olympus IX 71 Inverted Research Microscope (Olympus Corporation, Shibuya-ku, Tokyo, Japan) equipped with Narishige ON-2 Microinjector (Narishige CO. LTD., Setagaya-ku, Tokyo, Japan).

In both cases, ejaculated samples were allowed to be liquefied, washed in bicarbonate buffered commercial sperm wash media (Sperm Preparation Medium, Medicult, Denmark), and layered on 95% discontinuous density gradient medium carefully (Suprasperm, Medicult, Denmark). After 20 minutes of centrifugation at  $300 \times g$ , pellet was aspirated and washed twice in sperm wash media, resuspended to the desired volume and placed in the sperm droplet of the ICSI dish. ICSI was performed after aggressive sperm immobilization.

In the first case, 6 mature oocytes were injected after mechanical oocyte activation using a previously de-

scribed technique [19]. Briefly, plasma membranes of the oocytes were broken using a microneedle to maintain a  $Ca^{2+}$  influx and after a short period, the ICSI was carried out. Two oocytes were fertilized (33.3%) and cleaved to two 4-cell grade I embryos without any fragmentation and granulation with even blastomeres on day 2. These embryos were transferred on day 2 after mechanically assisted hatching had been performed. The second case had 11 mature oocytes and these oocytes were injected without oocyte activation. Only one oocyte was fertilized (9.1%) and one 4-cell grade I embryo without any fragmentation or granulation with even blastomeres transferred after mechanical assisted hatching on day 2. Luteal phases were supported by vaginal suppositories of micronized progesterone 600 mg/day t.i.d. (Progestan, Kocak, Turkey). A singleton clinical pregnancy was obtained in the first attempt in both couples. Pregnancies had uncomplicated clinical courses and resulted in delivery of two healthy female infants at 38 weeks and 38.5 weeks respectively. Table 1 shows the patient and cycle characteristics.

### 3 Discussion

Teratozoospermia is a common phenotype with several deformations ranging from cellular to subcellular levels in the head and tail of spermatozoa [15]. This condition is frequently associated with infertility and usually ICSI is used as the treatment of choice. However, severe sperm morphological abnormalities may result in poor

Table 1. Cycle characteristics of patients with globozoospermia. ICSI, intracytoplasmic sperm injection.

	Couple I (ICSI with oocyte activation)	Couple II (ICSI without oocyte activation)
Male age (years)	35	31
Female age (years)	37	26
No. of oocytes retrieved	10	11
No. of mature oocytes	6	11
No. of fertilized oocytes	2	1
Fertilization rate (%)	33.3	9.1
Cleavage rate (%)	100	100
No. of embryos transferred	2	1
Clinical pregnancy	+	+
Sac number	1	1
Implantation rate (%)	50	100
Livebirth	1 (Female)	1 (Female)

fertilization rates and embryo quality even with ICSI application [16]. Many studies reported various low fertilization rates with complete globozoospermic samples and total fertilization failures were observed in some cases [9]. Previous studies showed that the majority of the oocytes injected by round-headed acrosomeless spermatozoa remain intact and cannot complete second meiotic division, probably because of the inability of the round-headed sperm to activate an oocyte as a result of premature chromatin condensation [12,17].

Globozoospermia, which is a pathology of acrosome, probably shows X-linked, sex-restricted dominant, or autosomal recessive modes of inheritance [2], but so far, no responsible genes have been identified. Recently, murine studies revealed some hereditary information on severe teratozoospermia and globozoospermia [3]. Since these patients display normal haploid, sex chromosome and aneuploidy status, ICSI can be conceivably offered as a treatment for their infertility [18]. There have been several reports of healthy offsprings resulting from ICSI applications with round-headed spermatozoa [9–11]. Our cases were counseled under the light of the published literature and were informed about the elevation of aneuploidy rates [4] and possible inheritance of globozoospermia leading to infertility in the male offspring. Both cases decided to undergo ICSI treatment.

In familial globozoospermia, pregnancies and one livebirth were achieved previously without oocyte activation in repeated ICSI treatment cycles [11]. One of our cases underwent oocyte activation procedure together with ICSI and achieved a livebirth without repeated ICSI treatment cycles in familial globozoospermia.

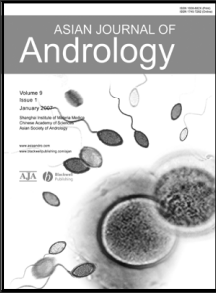


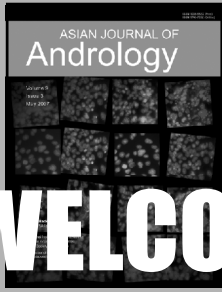
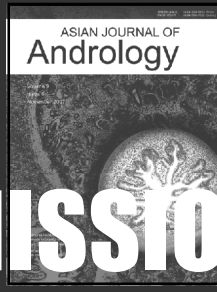
It was previously reported that oocyte activation prior to ICSI does not result in better fertilization rates [19]. On the contrary, it was suggested that assisted oocyte activation enables normal fertilization and pregnancy in cases with sperm and oocyte related fertilization failure caused by the impairment of the oocyte activation [14]. Mechanical oocyte activation in our study yielded a better fertilization rate and two good-quality 4-cell embryos were obtained in this case. The second case without oocyte activation had only one fertilized oocyte, although more oocytes were injected. Oocyte activation in this case was not performed because of the availability of more than 10 mature oocytes at that time but a low fertilization rate was achieved probably because of abnormal chromatin condensation [12] or centrosomal dysfunction [13].

In conclusion, this report supported the genetic origin of globozoospermia with a familial occurrence in two brothers. Although the fertilization rate was still low after ICSI, current ICSI procedures may overcome the infertility associated with globozoospermia and result in normal healthy livebirth with and without assisted oocyte activation. In our cases, oocyte activation before ICSI resulted in a better fertilization rate, and provided more fertilized oocytes.

## References

- 1 Castellani L, Chiara F, Cotelli F. Fine structure and cytochemistry of the morphogenesis of the round headed sperm. *Arch Androl* 1978; 1: 291–7.
- 2 Stanislavov R, Ganev V. A clinico-genetic study of male infertility with globozoospermia. *Akush Ginekol (Sofia)* 1998; 37: 20–2.
- 3 Juneja SC, van Deursen JM. A mouse model of familial oligoasthenoteratozoospermia. *Hum Reprod* 2005; 20: 881–93.
- 4 Carrell DT, Wilcox AL, Udoff LC, Thorp C, Campbell B. Chromosome 15 aneuploidy in the sperm and conceptus of a sibling with variable familial expression of round-headed sperm syndrome. *Fertil Steril* 2001; 76: 1258–60.
- 5 Vicari E, Perdichizzi A, De Palma A, Burrello N, D'Agata R, Calogero AE. Globozoospermia is associated with chromatin structure abnormalities: case report. *Hum Reprod* 2002; 17: 2128–33.
- 6 Ito C, Suzuki-Toyota F, Maekawa M, Toyama Y, Yao R, Noda T, *et al.* Failure to assemble the peri-nuclear structures in GOPC deficient spermatids as found in round-headed spermatozoa. *Arch Histol Cytol* 2004; 67: 349–60.
- 7 Escalier D. Failure of differentiation of the nuclear-perinuclear skeletal complex in the round-headed human spermatozoa. *Int J Dev Biol* 1990; 34: 287–97.
- 8 Singh G. Ultrastructural features of round-headed spermatozoa. *Int J Fertil* 1992; 37: 99–102.
- 9 Liu J, Nagy Z, Joris H, Tournaye H, Devroey P, Van Steirteghem A. Successful fertilization and establishment of pregnancies after intracytoplasmic sperm injection in patients with globozoospermia. *Hum Reprod* 1995; 10: 626–9.
- 10 Stone S, O'Mahony F, Khalaf Y, Taylor A, Braude P. A normal livebirth after intracytoplasmic sperm injection for globozoospermia without assisted oocyte activation. *Hum Reprod* 2000; 15: 139–41.
- 11 Kilani Z, Ismail R, Ghunaim S, Mohamed H, Hughes D, Brewis I, *et al.* Evaluation and treatment of familial globozoospermia in five brothers. *Fertil Steril* 2004; 82: 1436–9.
- 12 Battaglia DE, Koehler JK, Klein NA, Tucker MJ. Failure of oocyte activation after intracytoplasmic sperm injection using round-headed sperm. *Fertil Steril* 1997; 68: 118–22.
- 13 Nakamura S, Terada Y, Horiuchi T, Emuta C, Murakami T, Yaegashi N, *et al.* Analysis of the human sperm centrosomal function and the oocyte activation ability in a case of

- globozoospermia, by ICSI into bovine oocytes. *Hum Reprod* 2002; 17: 2930–4.
- 14 Tesarik J, Rienzi L, Ubaldi F, Mendoza C, Greco E. Use of a modified intracytoplasmic sperm injection technique to overcome sperm-borne and oocyte-borne oocyte activation failures. *Fertil Steril* 2002; 78: 619–24.
  - 15 Toshimori K, Ito C. Formation and organization of the mammalian sperm head. *Arch Histol Cytol* 2003; 66: 383–96.
  - 16 Kahraman S, Akarsu C, Cengiz G, Dirican K, Sözen E, Can B, *et al.* Fertility of ejaculated and testicular megalohthead spermatozoa with intracytoplasmic sperm injection. *Hum Reprod* 1999; 14: 3726–30.
  - 17 Rybouchkin A, Van der Straeten F, Quatacker J, De Sutter P, Dhont M. Fertilization and pregnancy after assisted oocyte activation and intracytoplasmic sperm injection in a case of round-headed sperm associated with deficient oocyte activation capacity. *Fertil Steril* 1997; 68: 1144–7.
  - 18 Viville S, Mollard R, Bach ML, Falquet C, Gerlinger P, Warter S. Do morphological anomalies reflect chromosomal aneuploidies? Case Report. *Hum Reprod* 2000; 15: 2563–6.
  - 19 Kahraman S, Polat G, Sözen E, Akarsu C, Özbiçer T, Dirican K. Oocyte activation before intracytoplasmic sperm injection does not improve the fertilization and embryo quality. *European Society of Human Reproduction Andrology and Embryology, 15th Annual Meeting, 1999 June 26–30; Tours, France.*

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