Asian Journal of Andrology (2010) 12: 284–286
 1008-682X/09 $ 32.00
www.nature.com/aja

Letters to the Editor

"One sperm, one embryo, one baby"?

Chi Christina Ding[1], Kok Joo Thong[2]

[1]*IVF Unit, Reproductive Medicine Unit, Liverpool Women's Hospital, Liverpool* L8 7SS, *UK*
[2]*Royal Infirmary of Edinburgh - Assisted Conception Unit, Edinburgh EH16 4SA, Scotland, UK*

Asian Journal of Andrology (2010): 284–286. doi: 10.1038/aja.2009.72; published online 23 November 2009

Correspondence to: Dr Chi Christina Ding, IVF Unit, Reproductive Medicine Unit, Liverpool Women's Hospital, Crown Street, Liverpool L8 7SS, UK. Fax: +44–151–702–4342 E-mail: cding@doctors.net.uk
Received: 17 June 2009 Revised: 13 July 2009 Accepted: 24 September 2009 Published online: 23 November 2009

Dear Editor,

The introduction of intracytoplasmic sperm injection (ICSI) technology [1] has revolutionised the treatment of patients with severe oligozoospermia and azoospermia. A patient is usually considered oligozoospermic if his sperm concentration is less than 20 million mL^{-1}. A patient is labelled azoospermic according to the World Health Organisation guideline [2], if, on two different occasions, no spermatozoa can be detected by high-power microscopy after the seminal fluid has been centrifuged for 15 min at a centrifugation force of 3 000 × *g* or greater [3, 4]. Occasionally, it may be difficult to draw the line between azoospermia and severe oligozoospermia. About 10% of apparent azoospermic patients may have cryptozoospermia, that is, some spermatozoa may still be found on the day of ovum retrieval from a careful search in their ejaculate [5]. However, cryptozoospermia is often intermittent and the recovery of spermatozoa from the ejaculate of cryptozoospermic patients is far from guaranteed. Occationally, extreme oligozoospermia may be misdiagnosed as azoospermia.

In theory, one sperm is all that is needed to fertilise an egg and achieve a successful pregnancy using ICSI technique. For most (moderate) oligozoospermic patients, sperm are retrieved successfully from their ejaculated samples collected on the day of egg retrieval. On the other hand, testicular sperm extraction-testicular biopsy (TESE) is the essential technique in recovering sperm in azoospermic patient for use in ICSI. TESE is genuinely regarded as the last resort if no sperm can be retrieved from the ejaculated samples of severe oligozoospermic or cryptozoospermic patients. This belief, however, is challenged by a case we encountered in our clinical practice.

We hereby report the birth of a healthy baby using the one and only motile spermatozoon recovered 24 h before ICSI from the ejaculate of a man with nonobstructive intermittent azoospermia (secondary to cryptorchidism/orchidopexy). No sperm were recovered from a fresh TESE or the ejaculated sample on the day of the ICSI.

A married couple were initially referred to our Assisted Conception Unit for ICSI treatment in 1999. They presented with a 2 years' history of primary infertility secondary to severe male factor of nonobstructive origin. The husband was a 35-year-old man with a past history of cryptorchidism and bilateral orchidopexy. Both his testicular volumes were 10 mL. Analyses of his semen samples showed profound nonobstructive oligozoospermia with intermittent azoospermia. His total sperm count varied from 0 to 0.27 million mL^{-1} (typically < 0.07), motility ranged from 0% to 60% (typically < 20%), and typical volume was around 4 mL. He had a raised follicle stimulating hormone level of 19.7 mIU mL^{-1}, with normal karyotype and testosterone levels.

His wife was 23 years old with regular ovulatory

cycles and no identifiable factors contributing to infertility.

The couple was counselled regarding the need for ICSI treatment in view of the quality and quantity of sperm in the ejaculate.

They underwent three full ICSI cycles and one natural thaw cycles between May 2000 and September 2001 without achieving a clinical pregnancy. She responded well in all treatment cycles with around 9–13 oocytes recovered and more than 50% fertilised in each cycle. Despite the poor quantity and quality in all his sperm samples, enough motile spermatozoa were produced each time to achieve reasonably good fertilisation rate. In April 2002, they achieved a successful pregnancy in their fourth ICSI cycle. She had a healthy baby girl of 3.6 kg delivered at 39 weeks gestation.

Significant deterioration in his sperm quality and quantity had occurred by the time they returned for their fifth ICSI attempt in April 2004. One of the two ejaculated sperm sample for pretreatment assessment was azoospermic. The couple was made aware that the poor quality and quantity of the sperm excluded the possibility of sperm cryopreservation, as it was unlikely that viable sperm would survive the thawing process. The couple consistently ruled out the option of using donor sperm as standby, while being fully aware that there was a possibility that an azoospermic sample may be produced on the day of oocyte retrieval.

Three more sperm samples were collected during the course of this treatment cycle. One ejaculated sperm sample was collected 24 h before the oocyte recovery. Although this is not a part of the unit's protocol, in view that the couple refused to have donor sperm backup, this additional sperm sample was taken as the backup. It is in fact from this sample that one motile and morphologically normal sperm was found and injected, which eventually resulted in a successful pregnancy and delivery. Three additional nonmotile sperms with normal morphology and signs of vitality were also found from this sperm sample and injected, but none of these achieved successful fertilisation.

The sperm samples were prepared by putting them on top of layered mini gradients (Sil-select, Microm UK Ltd, Bicester, UK) and centrifuged for 30 min at 100 × *g*. and then washed at 5 min at 400 × *g*. The sperm sample at the bottom of the tube—the pellet (0.1 mL)—was spread into medium (medicult, Origio Ltd, Reigate, UK) droplets in a Petri dish covered with paraffin oil. The sample was then cultured in the incubator overnight in 5% CO_2 at 37 ºC.

On the day of the oocyte retrieval procedure, two more sperm samples (one fresh ejaculate and one surgical testicular sperm retrieval/TESE) were collected, but both were azoospermic.

Successful fertilisation was confirmed 18 h later. A four-cell grade II embryo was replaced into the endometrium 2 days later. A healthy boy was delivered by caesarean section at 40 weeks gestation, weighing 4 110 g in January 2005.

One of the interesting points of this case is that the only motile sperm obtained came from the ejaculate rather than from TESE. This fact highlights the importance of a thorough and careful search of the ejaculatory sperm samples in severely oligozoospermic patients. To a certain extent, it also reflects some of the limitations of TESE. It is known that focal spermatogenesis occurs in nonobstructive oligozoospermic men. In addition, increasing evidences have shown that only part of the testes of these men have mature spermatozoa [6, 7]. With no means to localise the sites of spermatogenesis and determine the optimal site for sampling, randomly taking a small fraction of the testis may explain why its success rate in sperm recovery is not as high as may be expected [8].

Another interesting point is that all the sperms (motile or nonmotile) recovered were from the ejaculatory sample taken 24 h before, rather than the routine ejaculatory sample taken on the day of the egg recovery.

If the earlier ejaculatory sample were not taken, then no sperm would have been available for ISCI on the day of egg collection. From a physiological point of view, it is a matter of probability whether a particular sperm is present in an ejaculatory sample. Therefore, multiple ejaculated samples with short abstinence period may have improved the chance of successful sperm retrieval. This is yet to be confirmed by a dedicated study as it would be difficult to conduct a randomised controlled study on whether repeat ejaculation would increase the chance of sperm retrieval from the ejaculate because of the rarity of intermittent azoospermic/cryptozoospermic cases. Nevertheless, since this case, the method of using multiple ejaculated sperm samples has been adopted in our unit for similar clinical scenarios.

In summary, this letter highlights a couple of important issues in the management of intermittent azoospermic/cryptozoospermic patients. First, it is essential to thoroughly examine ejaculatory samples, not just simply rely on invasive surgical sperm retrieval techniques. Second, multiple ejaculatory samples with short abstinence period may improve the chance of

successful sperm retrieval.

References

1 Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 1992; 340 : 17–8.
2 World Heath Organization (1999) Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction, 4th edn. Cambridge: Cambridge University Press.
3 Corea M, Campagnone J, Sigman M. The diagnosis of azoospermia depends on the force of centrifugation. Fertil Steril 2005; 83: 920–2.
4 Report on Evaluation of the Azoospermic Male. American Urological Association/American Society for Reproductive Medicine Practice Committee Report; 2001.
5 Lindsay KS, Floyd I, Swan R. Classification of azoospermic samples. Lancet 1995; 345:1642.
6 Tournaye H, Camus M, Goossens A, Liu J, Nagy P, *et al*. Recent concepts in the management of infertility because of non-obstructive azoospermia. Hum Reprod 1995; 10 (Suppl 1):115–9.
7 Hauser R, Botchan A, Amit A, Ben Yosef D, Gamzu R, *et al*. Multiple testicular sampling in non-obstructive azoospermia—is it necessary? Hum Reprod 1998; 13: 3081–5.
8 Vernaeve V, Krikilion A, Verheyen G, Van Steirteghem A, Devroey P, *et al*. Outcome of testicular sperm recovery and ICSI in patients with non-obstructive azoospermia with a history of orchidopexy. Hum Reprod 2004; 19: 2307–12.