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LETTER TO THE EDITOR

Pregnancy outcome after intracytoplasmic sperm injection with strontium oocyte activation in a globozoospermic patient

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Dear Editor,

I am Dr Xiao-Yu Yang, from the Center of Clinical Reproductive Medicine in the First Affiliated Hospital at the NanJing Medical University, Nanjing, China. We present here a case report of a globozoospermic patient whose partner became pregnant after intracytoplasmic sperm injection (ICSI) with assisted oocyte activation (AOA). Globozoospermia is characterized by the presence of 100% round-headed spermatozoa lacking an acrosome. The lack of the acrosome, which renders spermatozoa unable to bind to the zona pellucida or fuse with the oocyte oolemma, is considered to be the cause of infertility in these patients.[1]

The introduction of ICSI provided a possible solution for patients suffering from globozoospermia. Since then, many cases of successful pregnancies after ICSI with globozoospermic sperm have been reported;[2,3] however, the fertilisation rate after ICSI in these cases has been low, and some patients failed ICSI treatment because of limited or failed fertilisation.[1] Fertilisation failure was initially attributed to the reduced ability of globozoospermic sperm to activate the oocyte.

Rybouchkin *et al.*[4] demonstrated that the fertilisation rate was improved by the addition of a calcium ionophore. After that, ICSI combined with AOA was effectively used in many studies to improve the fertilisation rate and embryo development and to achieve live births from globozoospermic sperm from men with previously failed fertilisation attempts. Various methods of AOA, including the use of a calcium ionophore and electrical and mechanical stimuli, have been reported to be effective for fertilisation in men with globozoospermia.[3,4]

Strontium treatment, which is proven to induce calcium oscillations in mice, was found to be an effective method for AOA in cases of low or absent fertilisation after ICSI.[5–7] Whether strontium is effective at improving fertilisation by globozoospermic sperm has not been reported. We report a successful case of a twin pregnancy resulting in live births following ICSI and strontium oocyte activation with round-headed sperm.

A couple with primary infertility of 5 years' duration visited our centre. Both members of the couple were 27 years old and healthy, with no physical problems other than the husband's semen parameters. The wife had no fertility problems. Analysis of a semen sample showed normal values for volume (3.3 ml), sperm concentration (56×10^6 ml^{-1}) and motility (45%). The Diff–Quik staining method revealed that 100% of the spermatozoa were round-headed and lacking an acrosome[8] (**Figure 1**). Assessment of the acrosome with fluorescent *Pisum sativum* agglutinin staining of the human spermatozoa was also conducted[9] (**Figure 1**). Ultrastructural characteristics of the round-headed sperm were evaluated using a scanning electron microscope (JEOL Technics Ltd, Akishima-Shi, Tokyo, Japan)[3] (**Figure 1**). Spherical heads without acrosomes are shown. The karyotypes of the couple were obtained from peripheral lymphocytes and were 46,XX in the wife and 46,XY in the husband.

A conventional long protocol was used to stimulate follicular development. Two cycle attempts were conducted. In the first cycle, the couple decided to undergo the conventional ICSI procedure. ICSI was performed on 20 metaphase II oocytes. Following ICSI, four oocytes were normally fertilized. Three days after insemination, two embryos reached the four-cell stage and one reached the three-cell stage without fragmentation; one did not cleave. Two embryos in the four-cell stage were transferred; however, pregnancy did not occur.

After the failure of the first ICSI cycle, the couple decided to conduct $SrCl_2$ oocyte activation on the retrieved oocytes as part of the next attempt. Only three reports involving four patients undergoing $SrCl_2$ for human oocyte activation have been published, making $SrCl_2$ oocyte activation highly experimental. The risks of the treatment were explained in detail, and informed consent was obtained from the couple. The study protocol was approved by the local ethics committee of the First Affiliated Hospital of NanJing Medical University.

The second attempt was conducted 10 months after the first one using the same long ovarian stimulation protocol. Fifteen metaphase II oocytes were obtained and were randomly divided into two groups. Ten oocytes (Group B) underwent ICSI treatment combined with

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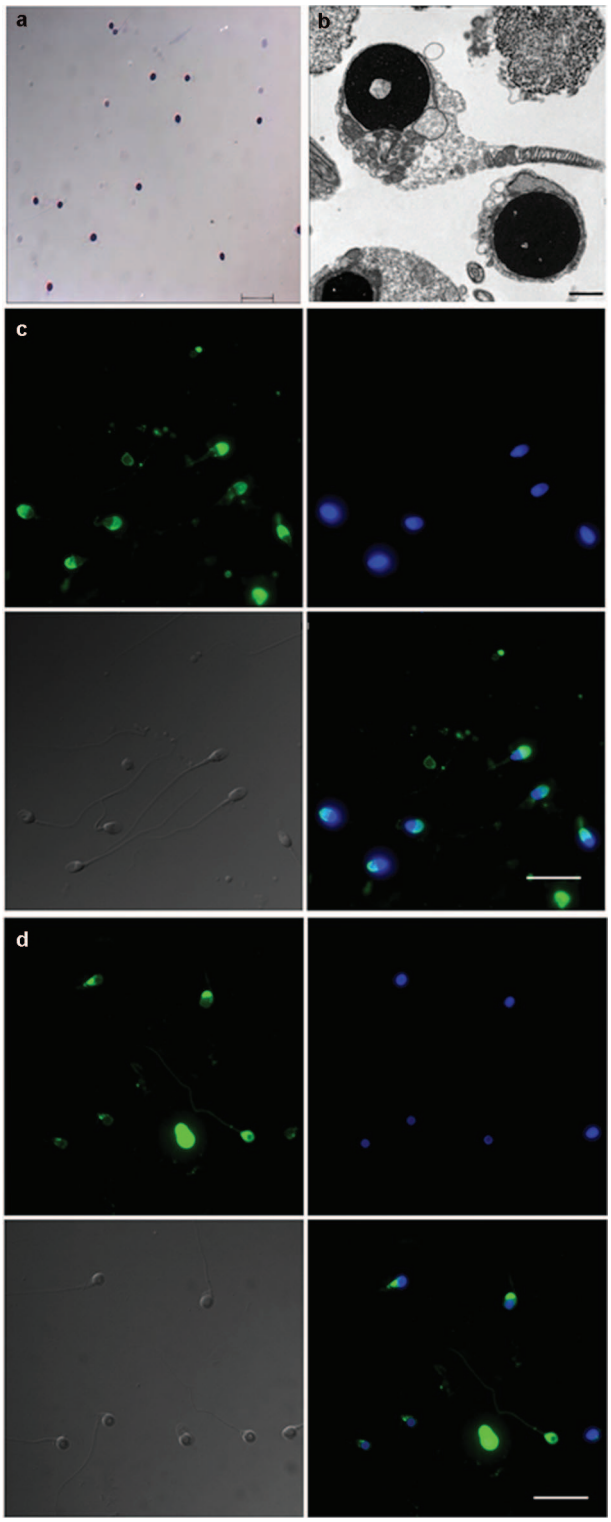

Figure 1 Round-headed sperm morphology in the globozoospermic patient. (**a**) Round-headed sperm morphology under a light microscope (scale bar=10 μm); (**b**) scanning electron micrograph of round-headed spermatozoa (scale bar=1 μm); (**c**) normal human spermatozoa fluorescence image after PSA staining (scale bar=10 μm); (**d**) round-headed spermatozoa fluorescence image after PSA staining (scale bar=10 μm). PSA, *Pisum sativum* agglutinin.

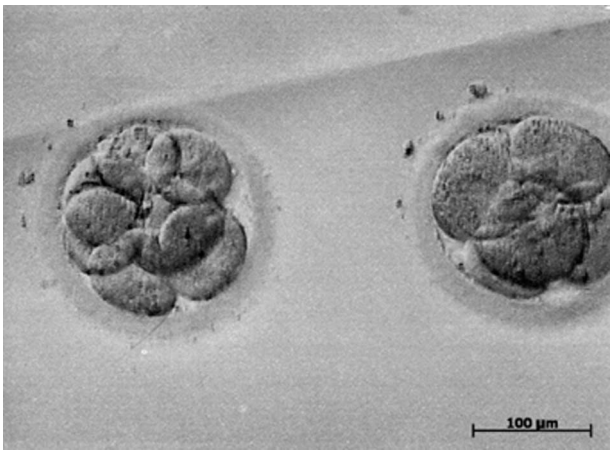

Figure 2 Image of day 3 embryos after $SrCl_2$ activation under a light microscope (scale bar=100 μm). The left embryo reached the eight-cell stage with no fragmentation; the right one reached the five-cell stage with 5% fragmentation.

$SrCl_2$ oocyte activation, and the other five (Group A) underwent ICSI only. After ICSI, the oocytes of Group A were activated in calcium-free human tubal fluid medium (SAGE BioPharma, Bedminster, NJ, USA) containing 10 mmol l^{-1} $SrCl_2$ (Sigma-Aldrich, Madrid, Spain) for 10 min at 37 °C and 5% CO_2. Following AOA, the oocytes were rinsed thoroughly in *in vitro* fertilisation medium (Medicult, Maaloev DK-2760, Denmark) and transferred to P1 medium containing 10% (v/v) serum substitute supplement and incubated at 37 °C under 5% CO_2.

One oocyte from Group A and two oocytes from Group B were normally fertilized. Three days after microinjection, the one embryo from Group A reached the four-cell stage with 5% fragmentation, while one of the two embryos from Group B reached the eight-cell stage with no fragmentation, and the other one reached the five-cell stage with 5% fragmentation. The two embryos (**Figure 2**) that underwent $SrCl_2$ activation were transferred. Two healthy female infants without any congenital abnormalities were delivered at 37 weeks gestation by caesarean section. **Table 1** shows the patient and cycle characteristics.

AOA is used to induce calcium oscillations in oocyte activation. AOA methods, such as the popular calcium ionophore method and the method of electrostimulation, mostly result in a single transient increase of intracellular calcium in the oocytes.[1] $SrCl_2$, unlike most of the AOA methods, was reported to cause oocyte activation accompanied by Ca^{2+} oscillations in mice.[7] Zhang *et al.*[10] speculated that Sr^{2+} probably promotes Ca^{2+} oscillations in mouse oocytes and embryos by sensitising the IP3 receptors (the Ca^{2+} release channels)

Table 1. Characteristics of the two cycles with globozoospermic sperm. (female: 27 years; male: 27 years)

	First Cycle	*Second Cycle*	
$SrCl_2$ activation		*Group A*	*Group B*
No. of mature oocytes	20	5	10
No. of fertilised oocytes	4	1	2
Fertilisation rate (%)	20	20	20
Cleavage rate (%)	75	100	100
No. of embryos transferred	2	No	2
Clinical pregnancy	No	No	Yes
Implantation rate (%)	No	No	100
Live birth	No	No	2 (female)

[a] These oocytes were activated with 10 mmol/L $SrCl_2$ for 10 min after microinjection.

to release Ca^{2+}. The efficiency of $SrCl_2$ for oocyte activation varies depending on the species.[5] It is most effective in mice, but in human oocytes, calcium oscillation has not been observed, which is the key to oocyte activation. Four case reports have been published demonstrating improved fertilisation rates and embryo quality following strontium was used for AOA in humans with low or no fertility, and successful pregnancies and live births have been achieved.[5–7]

Our study is the first reported case of successful pregnancy and live births after strontium was used for AOA with globozoospermic sperm. However, the fertilisation rate was not improved after strontium treatment, even though it seemed to improve the quality of the embryos. We could not confirm whether the twin pregnancy benefited from the strontium activation after ICSI. The potential toxic effects of AOA with strontium have not been fully determined in clinical ICSI, and the safety of strontium stimulus remains to be assessed.[6] Several studies have indicated that no physical or mental developmental disorders are associated with babies born after strontium stimulus, up to 12 months after birth.[5–7] However, there have not been any reports of genetic analysis in humans born following strontium stimulus treatment. The long-term effect of the potential toxicity of strontium on humans remains unknown.

In conclusion, oocyte activation using strontium for fertilisation with round-headed sperm may not be effective in increasing the fertilisation rate, even though the only improvement we measured was in embryo quality.

AUTHOR CONTRIBUTIONS

XQQ, XYY, WZ, ZMZ and JHS conceived and designed the study. XQQ completed the embryo work in the laboratory. JYL and YG completed the clinical work. YGC revised the manuscript for important intellectual content. XYY and JW wrote the manuscript, which was read and approved by all authors.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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