A comparison of the efficacy of intracytoplasmic sperm injection (ICSI) using ejaculated sperm selected by high magnification versus ICSI with testicular sperm both followed by oocyte activation with calcium ionophore

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Summary

Purpose: To determine in a couple where the male had globozoospermia with failed fertilization despite intracytoplasmic sperm injection (ICSI) if fertilization could be achieved by using high magnification ICSI or by oocyte activation by calcium ionophore.

Methods: Twenty eggs were retrieved and divided according to source of sperm, i.e., ejaculated by testicular aspiration or with donor sperm. Following ICSI the eggs were exposed to calcium inophore within one hour. The sperm were magnified 6300x in an attempt to find some sperm with evidence of at least some acrosome.

Results: None of the seven eggs inseminated by ICSI with ejaculated sperm or the seven inseminated with ICSI with testicular sperm fertilized even with attempted oocyte activation with calcium ionophore. However, four of the six oocytes that were inseminated with donor sperm did fertilize. None of the round-headed sperm showed any partial acrosome even using high magnification ICSI.

Conclusions: It is possible that for successful fertilization with round-headed sperm there has to be at least enough acrosomal material to make some sperm-associated oocyte activating factor.

Kew words: Globozoospermia; High magnification ICSI; Calcium ionophore.

Introduction

Liu *et al.,* showed that fertilization and pregnancies were possible following intracytoplasmic sperm injection (ICSI) for women whose male partners had globozoospermia [1] despite multiple previous failures in other studies using conventional insemination. Stone et al and Kilani *et al.* also reported a similar success [2, 3]. A subsequent study by Battaglia *et al.* found that even with ICSI sometimes round-headed sperm fail to induce oocyte activation [4].

Fertilization and a successful pregnancy have been reported in the female partners of some men with globozoospermia with deficient oocyte activation capacity by assisted oocyte activation with calcium chloride and ionophore [5].

The present study compared testicular vs ejaculated round-headed sperm in allowing fertilization of oocytes from a woman who previously failed to have any of 14 eggs fertilize by ICSI. In both cases the process would be aided by calcium ionophore to allow oocyte activation and the use of high magnification (6300x) to theoretically better select the sperm with maybe some acrosomal material [6].

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Materials and Methods

The metaphase II oocytes would be equally divided into three methods of fertilization: 1) ICSI with round-headed sperm obtained by testicular aspiration on the day of oocyte retrieval, 2) ICSI with ejaculated round-headed sperm, and 3) ICSI with donor sperm.

The globozoospermic sperm used for fertilization were selected by high magnification ICSI 6300x (Nomarski Optics).

Seven oocytes were inseminated through ICSI with round-headed sperm obtained by testicular aspiration on the day of oocyte retrieval. A 0.8 ul drop of sperm was placed in 5 ul drop of PVP (Conception Technologies, La Jolla, CA) covered with mineral oil for assisted reproductive technology (ART) (Irvine Scientific) in a Willco glass bottom dish (Precision Instruments). The Nikon TE-2000 inverted microscope with Nomarski optics and the camera Imagen/Omnex model 12503-1 connected to a Sony video monitor were used in the sperm selection procedure. Sperm was analyzed at high magnification of 1000x in oil immersion. The morphological assessment was conducted on the monitor screen which reached a real magnification of 6300x. The sperm was morphology selected with particular regard to the shape and presence of the acrosome, presence of vacuoles, and shape of the nucleus. Number of normal and abnormal sperm were noted as percentage. The sperm with the closest to normal parameters were immobilized, and then moved into another 5 ul PVP drop with the aid of an ICSI needle (Humagen). ICSI was performed as per the usual protocol and the oocytes were treated with calcium ionophore as described below. The oocytes were then incubated as usual.

Following ICSI, calcium ionophore activation of the oocyte

was attempted. Another seven oocytes were inseminated through ICSI with ejaculated round-headed sperm. Within one hour of injection the oocytes were exposed to 10 umol/l of calcium ionophore A23187 (Sigma, St. Louis, MO) in fertilization medium (Sage BioPharma Bedminster, NJ) with 10% serum protein substitute (Sage BioPharma, Bedminster, NJ) covered with mineral oil for ART (Irvine Scientific) for seven minutes at 37°F in 5.5 CO_2. The oocytes were then washed two times in fresh medium and incubated further as usual.

Results

There were 25 oocytes retrieved and 20 of the oocytes were metaphase II.

The outcome of ICSI with the three different sources of sperm is shown in Table 1. Only donor sperm fertilized the eggs (66.7%). Despite using high magnification ICSI not one sperm could be found that had any trace of the acrosome.

Table 1. — *Effect of ICSI with round-headed sperm.*

Source of sperm	# of eggs using ICSI	# of eggs fertilized	% fertilized
Ejaculated	7	0	0
Testicular	7	0	0
Donor	6	4	66.7

Conclusion

There are some cases where ejaculated sperm does not fertilize but testicular sperm is successful. However, this may not apply to round-headed sperm where probably what one sees in the ejaculate is also found in the testicle.

The probable explanation why this couple failed to fertilize with the husband's sperm despite other successful cases reported may be that in the other cases there was still some acrosome present. Using high magnification ICSI we were hoping that in some men with round-headed sperm that there might be some sperm with a little more acrosome than others and these would be the sperm to choose. At least in this case it appeared to be completely absent.

The fact that 66.7% of the eggs fertilized by donor sperm shows that there also was not a problem with defective oocytes also. The fact that some cases of globozoospermia have been reported where success with ICSI alone was achieved suggests there was enough acrosome present which was sufficient to produce some sperm-associated oocyte activating factor (SAOAF).

Some cases fail with ICSI alone but respond to calcium ionophore oocyte activation. The possible explanation for this observation is that even with sperm with no part of the acrosome present fertilization can be achieved by artificial oocyte activation with calcium ionophore.

This case demonstrates that calcium ionophore activation is not always successful whether using ejaculated or testicular sperm. By high magnification ICSI we could find no trace of any acrosome. Perhaps those cases where calcium ionophore 30 minutes after ICSI was successful with round-headed sperm could be explained by the presence of a small amount of acrosome that is still making some essential proteins needed for fertilization.

It would be interesting to examine by high magnification the round-headed sperm of males who have fertilized by ICSI alone vs ICSI with calcium ionophore to see if the outcome could be predicted. Furthermore it would be of interest to determine in the sperm of these males who can fertilize with ICSI alone whether the amount of acrosome is uniform or if some sperm have more acrosome than others. Perhaps some males who fail by ICSI alone but have had success with ICSI followed by calcium ionophore may have a minority of sperm with some acrosomal material that can be detected by high magnification which may allow fertilization with ICSI alone with calcium ionophore activator.

At present one method to determine if ICSI alone can fertilize vs the need for oocyte activation with calcium ionophore is to inject into mature mouse eggs and look for 2-cell formation (mouse oocyte activation test or MOAT) first without artificial oocyte activation with calcium ionophore [7]. If there is failure to fertilize, then subsequently calcium ionophore could be utilized.

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