Multiple intravenous oxytocin injection may yield sperm in azoospermic men scheduled for TESE

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ABSTRACT

Objective: The aim of the present trial is to evaluate the value of multiple IV injection of oxytocin in cryptospermic men scheduled for TESE/ICSI
Design: a prospective clinical trial
Materials and methods: Twenty eight infertile cryptospermic men were injected with 1.0 IU oxytocin IV daily for four successive days. Last injection was 20 minutes before collecting semen sample by masturbation. Data were then compared to their semen analysis one week before oxytocin injection (every case was its own self control).
Results: Motile spermatozoa were found in semen in 8 participants (28.6%). Number of sperm identified ranged between 1-5 motile sperm. Motility ranged from shaking movement to progressive movement. In cases where sperm showed any indication of movement, it was used for ICSI. TESE were performed in other subjects who yielded zero sperm. No difference in Blood pressure and pulse were recorded pre- and one hour post- injection. However, 5 cases complained of tension headache and hot flushes.
Conclusion: Multiple dose oxytocin injection in azoospermic men prior to ICSI may yield some spermatozoa, hence saving some cryptospermic men doing TESE.

Key words: oxytocin, azoospermia, cryptozoospermia, TESE

Oxytocin receptors were identified on Leydig and Sertoli cells of the testis, on epithelial cells throughout the epididymis, on peritubular smooth muscle cells in the cauda epididymis, and on the epithelial cells and circular smooth muscle layer of the ductus deferens (1). Oxytocin has been shown to increase contractility in the epididymis and to modulate steroidogenesis (2). In a concentration of 10 IU/ml, oxytocin was also found to significantly increase the percentage of motile spermatozoa and sperm velocity compared with saline controls (3).

Both non-obstructive azoospermia and cryptozoospermia (ocult / intermittent) represent the extremes of the spectrum of severe testicular failure. However, a recent cross over randomized controlled trial showed a lack of effect of single IV dose of oxytocin 3 IU in 49 severely oligozoospermic men (4). This could be due to real lack of effect of oxytocin or due to insufficient administration whether in dose or frequency or it could be that semen sample was collected before oxytocin exerts it full action. If there would be an effect, then its clinical value would be clear for cryptospermic men scheduled to do TESE /ICSI. The aim of this prospective clinical trial is to evaluate the multiple IV injections of higher dose of oxytocin in recently diagnosed cryptospermic men.
MATERIALS AND METHODS

The present study was conducted in private IVF unit between June 2003 till February, 2004. Eligibility criteria for inclusion in the study included patients with severe oligospermia in whom no sperm were found on at least two successive previous sperm examination; and patients diagnosed as suffering from non-obstructive azoospermia. Exclusion criteria included patients with obstructive azoospermia, and patients with azoospermia due to known genetic abnormalities. Those presenting with congenital bilateral absence of vas deferens (CBAVD) were also excluded from the study.

All participants were documented for past medical history. All were examined regarding their genitalia especially testicular volume. All participants were asked to get a semen sample one week before estimated time for start of oxytocin. Thorough semen analysis for this sample was done to detect any sperm. If sperm was found, participant was excluded from the trial. Those found to be cryptospermic were included in the study and they were considered as their own self-control. All were injected with 1.0 IU oxytocin IV daily for four successive days (of sexual abstinence). Blood pressure and pulse were recorded pre- and one hour post-injection. Last injection was 20 minutes before collecting semen sample by masturbation (after 3-4 days abstinence). We used oxytocin (Novartis, Egypt).

The sample was allowed to liquefy in an incubator at 37°C for 30 min. We used the density gradient media (Spermgrade-100 Vitrolife Scandinavian IVF Science Sweden), which was layered in a sterile conical centrifuge tube marked with patient ID. Pipette 1.0 ml of 90% solution into the tube first and then slowly 1.0 ml of 45% solution on top of it. Finally, 1.0 ml of semen is gently layered on the top. The tubes were then centrifuged for 20 minutes at 1200 g. The two top layers were removed and no residues were left on the tube wall.

Transfer the sperm pellet with as little of the 90% solution as possible to a sterile conical tubes with 5-10 ml Ham's F 10. Centrifuge for 10 minutes at 300g. We discarded the supernatant and repeat the wash. After the second wash, the pellet was re-suspended in an appropriate volume of equilibrated IVF medium. The washed sample was then assessed for motility and concentration using standard methods (World Health Organization, 1999). Morphology of sperm was assessed using oil immersion with magnification of x1000 under bright-field illumination. The same investigator examined all samples. In cases where sperm showed any indication of movement, it was either used for ICSI or cryopreserved.

ICSI procedure

After, oocyte collection, oocytes were denuded with hyaluronidase (Scandinavia IVF Science, Gothenburg, Sweden) and mechanical pipetting. Mature (metaphase II) oocytes were identified by the presence of the first polar body. Only those oocytes that had extruded the first polar body (metaphase II oocytes) were microinjected. Immediately before injection, the sperm suspension was added to a 50 µl droplet of polyvinylpyrrolidone (PVP; Medicult). Oocytes were microinjected ~5 h after retrieval in microdroplets of IVF medium covered with lightweight paraffin oil. A single motile spermatozoon with apparently normal morphology was immobilized by touching its tail with the injection pipette and aspirated tail-first into the injection pipette. The sperm was microinjected into the ooplasm at the 3 o'clock position, the polar body being oriented at the 6 or 12 o'clock position.

Fertilization was assessed 24 h after injection if two pronuclei (2PN) were present and the second polar body had been extruded, then left in culture for a further 24 h.

Embryos were transferred 48-72 h after oocyte retrieval using Wallace embryo transfer catheter. Routinely, a maximum of four embryos were transferred to the patient. In few cycles, patients received five or six embryos due to repeated failure in previous cycles, advanced maternal age or poor quality embryos. Pregnancy was determined by serum HCG measurement on day 14-15 after transfer and clinical pregnancies were detected by presence of a gestational sac on ultrasound scans performed 6 weeks after embryo transfer. The implantation rate was defined as (the number of gestational sacs divided by the number of embryos transferred) x100.
RESULTS

Twenty eight infertile men diagnosed as azoospermia at allocation (confirmed by at least two recently centrifuged semen pellet analyses) were enrolled in the trial. Motile spermatozoa were found in semen in 8 participants (28.6%). Number of sperm identified ranged between 1-5 motile sperm. Motility ranged from shaking movement to progressive movement. There was no difference in ejaculate volume in these samples and those used to diagnose azoospermia. No difference in Blood pressure and pulse were recorded pre- and one hour post- injection. However, 5 cases complained of tension headache and hot flush. The results are displayed in table 1.

DISCUSSION

Oxytocin (OT) is a neurohypophysial hormone with a key role in the central regulation of penile erection (5). OT receptors (OTR) are present in human testis and epididymis and mediate its contractility and therefore is important in determining sperm transport. (6). In fact, endogenous oxytocin exists in the epididymis in similar concentrations in all regions. Our participants were patients in whom the chance of finding sperm in their semen was very remote proved by semen analysis just one week before start of our trial. The dosages and intervals from oxytocin administration to collection have varied widely between reports. Response to treatment has also varied between reports and with method of collection. We chose the dose of 0.5 IU/ml based on the observation that increasing the dose of oxytocin would result in a greater increase in both fluid output and the number of spermatozoa after administration of the oxytocin.

Unlike Byrne et al study, we asked our participants to commence semen collection 20 minutes after the last injection. This is supported by the observation of Nicholson and colleagues who were able to show that treatment with oxytocin caused an increase in sperm number and fluid volume starting 10 minutes after treatment. Values did not return to that of controls until 40 minutes after treatment. Administration of an oxytocin antagonist (des Gly-NH2d(CH2)5-[Tyr2,Thr4]OVT) had no immediate effects on sperm numbers or fluid flow but there was a significant reduction in both values 40-50 minutes after treatment. (7) Thus 20 minutes was thought to be a reasonable time to allow for sperm transfer.

This is further supported by other investigators who did not observe a change in sperm concentration when semen collection was 5 minutes after oxytocin (the same finding as Byrne et al study), but an increase in sperm concentration was found 10 minutes after oxytocin injection (8,9). The increase in sperm number following oxytocin administration has been attributed to an increase in smooth muscle contraction surrounding the epididymis, which enhances spermatozoa movement into the deferent duct.

The mechanism behind our findings is not fully understood. It may be that oxytocin acts directly on the contractile tissues of the seminiferous tubule and epididymis causing an increased rate of sperm passage to the epididymis and then to the deferent ducts. This is supported by the fact that sperm transport through the testicle from the site of spermiation in the seminiferous tubules through the testis till the epididymis is mediated by actions not inherent to the spermatozoa. Sperm cells do not gain the ability to propel themselves forward until they have traveled through the epididymis. Transport of non-motile spermatozoa is governed primarily by contraction of smooth muscle surrounding the seminiferous tubules (10). Thus the possibility of spermiation and passage through seminiferous tubules to be enhanced by oxytocin is appealing.

Table 1. Outcome of ICSI in those who yield sperm after oxytocin (Ejaculate group) and those did not (TESE group)

<table>
<thead>
<tr>
<th></th>
<th>Ejaculate group</th>
<th>TESE group</th>
<th>NS</th>
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<tbody>
<tr>
<td>Number of sperms retrieved</td>
<td>9.8 (2.9)</td>
<td>10.9 (4.0)</td>
<td></td>
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<tr>
<td>Oocytes</td>
<td>7.3 (2.5)</td>
<td>8.5 (3.7)</td>
<td></td>
</tr>
<tr>
<td>2 PN oocytes</td>
<td>4.8 (2.2)</td>
<td>6.2 (3.1)</td>
<td></td>
</tr>
<tr>
<td>Embryos transferred</td>
<td>3.8 (1.3)</td>
<td>4.1 (0.9)</td>
<td></td>
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<tr>
<td>Clinical P.R</td>
<td>2/8 (25%)</td>
<td>4/20 (20%)</td>
<td></td>
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<tr>
<td>Implantation rate</td>
<td>37.5%</td>
<td>40%</td>
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However, it is well known that passage of the spermatozoa through the epididymal ducts requires 12 days and the authors used the drug for only 4 days. On the other hand, the Leydig cells produce endogenous oxytocin (11). Depletion of testicular oxytocin by destruction of the Leydig cells can be associated with a decrease in spontaneous seminiferous tubule contractility. Contractility can be restored by the administration of exogenous oxytocin (12). Some may argue that the second ejaculate can be an alternative method to obtain spermatozoa in similar situations. However, this is only supported in the medical literature in cases of oligospermia and not cases of cryptozoospermia as in our study (13).

In conclusion, although this trial would be strengthened with a control group, one may assume that multiple oxytocin injection and sperm cryopreservation may prevent testicular biopsy or cycle cancellation in some cryptospermic men scheduled for TESE/ICSI. However, it is difficult to predict which patients might benefit from Oxytocin injection.

REFERENCES


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