Letters to the Editor

Testicular sperm aspiration (TESA): the appropriate technique

Dear Sir,

We read with interest the article by Friedler et al. (1997). They quoted low sperm retrieval rates when using testicular fine needle aspiration (TEFNA) (11%) compared with testicular sperm extraction (TESE) (43%). This has not been our experience. We believe that this is basically due to the difference in methodology of the fine needle aspiration of testicular tissue for sperm isolation. For simplicity, we describe the details of our technique below and then discuss the pitfalls and tips which might make the difference in success.

Testicular sperm aspiration (TESA) is performed as follows (Gorgy et al., 1997): the testis is stabilized between the thumb and the index and middle fingers and a 19 gauge butterfly needle is directed through the stretched skin of the scrotum into the testis. Negative pressure is created by attaching a 20 ml syringe to the plastic tubing of the butterfly needle and fully drawing back the plunger. The negative pressure is maintained by applying a pair of small artery forceps across the tubing, near the attachment of the syringe. The needle is moved backwards and forwards in rapid movements within the substance of the testis in different directions to sample different areas through the same puncture site. Aspirated testicular tissue biopsy is visible within the plastic tubing of the butterfly needle. The needle is withdrawn when enough or no more testicular tissue is aspirated. On withdrawal of the needle, the core of testicular tissue that stretches between the tip of the needle and the testis, is cut close to the scrotal skin. The 20 ml syringe is then replaced with a tuberculin (1 ml) syringe containing gamete culture medium. The aspirate is flushed with the culture medium, after releasing the artery forceps, into a sterile Eppendorf tube. This specimen is coarsely dissected into small sections using sterile fine surgical scissors and immediately examined under the microscope to check for the presence of motile spermatozoa. The same technique is repeated on the same or the other side until a sufficient specimen is obtained.

Further dissection is carried out in the laboratory using sterile 25 gauge needles in ~3 ml of gamete culture medium. The tissue is dissected such that the tubules themselves are visualized under the dissecting microscope and slit open longitudinally to release the spermatozoa. The macerated sample together with medium are placed in a 5 ml sterile Falcon tube and the large segments of tissue are allowed to settle for ~10 min. The supernatant is aspirated and then centrifuged for 10 min at 1000 g. The pellet is resuspended in ~0.2 ml of fresh gamete culture medium. The spermatozoa are directly recovered from the suspension (direct sperm aspiration) for intracytoplasmic sperm injection (ICSI; Craft et al., 1995).

Needle aspiration biopsy of the testis is found to correlate well with conventional open biopsy (Gottschalk-Sabag et al., 1993; Mallidis and Gordon-Baker, 1994) and is an effective alternative for the assessment of spermatogenesis (Craft et al., 1997) and it is recommended that local anaesthesia (LA) to be the standard method due to its low cost and better patient acceptability than open biopsy (Landau, 1996). On the other hand, multiple extensive testicular biopsies may represent a significant loss of testicular tissue (Gil-Salom et al., 1996). Friedler et al. used a 21 gauge needle rather than 19 gauge we recommend. They probably omitted the need for rapid vertical movement of the needle to cut and sample enough testicular tubules, and may not have sampled the stretched core of testicular tissue between the tip of the needle and the testes. The tissue was not further microdissected in the laboratory prior to centrifugation. Moreover, the specimens were subjected to discontinuous Percoll gradient centrifugation which is known to be associated with poor sperm recovery in such samples (Craft and Tsirigotis, 1995). However, further well controlled studies are needed to compare the efficacy of TESA and TESE, and the method used for needle sampling is critical. As the TESA technique samples many more different areas of the testis, it can be more successful than TESE, particularly in cases of non-obstructive azoospermia with focal spermatogenesis.

We hope we have clarified the appropriate way to perform TESA. We believe that if the same study is now repeated with the technique described there may not be a significant difference in sperm recovery rates between TESA and TESE (open biopsy). Currently we are planning a controlled study comparing TESA with TESE outcomes. If TESA is not successful, TESE will be carried out. This will determine whether there are cases of unsuccessful TESA where spermatozoa can be obtained by TESE. However, it will not determine cases where TESA is successful in TESE failures, since we do not consider it acceptable to randomize patients to TESE first in the light of overwhelming experience of success for TESA. We are confident that the result of this study will validate our opinion mentioned above.

References


Dear Sir,

We read with interest the letter by Gorgy et al. expressing concerns regarding our technique in performing testicular sperm aspiration (TESA). The assumptions made in the above letter concerning our methodology are unjustified. To clarify the issue allow us to make the following points.

Success in aspirating testicular spermatozoa by TESA is dependent upon three major factors: the actual status and extent of spermatogenesis in the patient’s testicles, the technique of sperm aspiration and the methodology of the laboratory procedures aiming to extract and identify sperm cells.

Methodology of TESA

First allow us to reassure the authors that our methodology of TESA has proven to be very efficient in our hands for the last 2 years, especially for patients with obstructive azoospermia requiring TESA. Actually, our methodology of sperm aspiration was based on the publications and presentations by Craft and Tsirigotis (1995). To clarify this, allow me to be specific, starting with the methodology of TESA. Contrary to the assumptions made in the above letter, we do perform carefully rapid vertical movement of the needle within the substance of the testis in different directions and do sample many sites in the stretched core of testicular tissue. We do cut the core of testicular tissue that stretches between the tip of the needle and the testis. Moreover, many times one can pull the testicular tissue using the small artery forceps until a long strip is retrieved and cut close to the skin. In our experience, in patients with non-obstructive azoospermia, these strips are fibrotic bands of testicular seminiferous tubules, which only rarely contain spermatozoa (unlike in obstructive azoospermia) and our impression is that a lot of tissue aspirated from mushy testicles actually serves as a bad sign linked with diminished chances to find any sperm cells.

Concerning the needle used for aspiration: we indeed use a 21 gauge butterfly needle. This concurs with the recommendations published by Craft’s group. Citing from the references given by Gorgy et al. it would appear that probably both needles are plausible and the difference between them may not be that crucial. Craft and Tsirigotis (1995), page 1624: ‘The TESA technique.... involves passing a 21–23 gauge butterfly needle directly into the testis ....;’ Craft et al. (1997), Materials and methods: line 7: ‘A 19 or 21 gauge Butterfly needle...’.

Furthermore, the tissue passed to the laboratory is microdissected if its size requires it.

Laboratory procedures

Concerning the laboratory methods used to find spermatozoa, we totally agree that in almost all cases no need exists to perform Percoll separation, and therefore we have stated it in our paper’s Materials and methods: ‘Cell separation .... by discontinuous Percoll gradient was not performed in all cases.’ Actually the mini-Percoll described was performed on only two occasions when a lot of cell debris was seen and we agree that this procedure might lead to loss of sperm cells. Our routine procedure is indeed centrifugation of the aspirate at 300 g for 10 min, then following red cell lysis continuing with a further wash and centrifugation for 5 min at 250 g, finally incubating the pellet in human tubal fluid (HTF)–HEPES–albumin culture medium prior to direct sperm aspiration. We apologize if the description of the laboratory manoeuvres was not clear enough in our paper. However, it must be stressed that the fine needle aspirates in our study were not subjected to Percoll in most cases. We do not believe that one centrifugation at 1000 g for 10 min as opposed to two centrifugations of 300 g for 10 min then 250 g for 5 min, may explain the differences in our findings (not that we have your exact data in patients with non-obstructive azoospermia).

Patient selection

Having answered all the concerns expressed in the letter, our impression that the differences in our methodologies are minor. The above considerations leave us the only plausible explanation for the differences in our experience, that is the difference in the patient’s state/extent of spermatogenesis between our groups. We would like to stress that the selection criteria in our study were rigorous, including histological proof of non-obstructive azoospermia. Moreover, all patients underwent extended sperm preparation of their ejaculate (procedure described in previous papers, Ron-El et al., 1997a,b) and all those with any spermatozoa found excluded from the study, leaving the more extreme cases of non-obstructive azoospermia. It seems to us that the main factor determining success in this particular clinical situation is the quantity of testicular tissue examined. We have no doubt that open biopsy allows to retrieve and examine more tissue than needle aspiration. Finally, one might wonder whether using a 19 gauge needle as you recommend should be considered as fine needle aspiration, taking into account that it is the needle used routinely for testicular biopsy in the past. Furthermore, we performed serial ultrasonographic and Doppler examinations to our patients following TESA and testicular sperm extraction (TESE) and found that in the short term even after TESA some changes may be demonstrated in the testicles and no significant changes were noted at 6 months following both procedures. No need to say that our work led to conclusions indeed performing fine needle aspiration with use of 21 gauge needle and not 19 gauge, which could be examined in a different study, if the difference in the quantity of the tissue

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aspirated with the 19 g needle is crucial. However, as open biopsy is performed quite easily and rapidly in our experience, we feel that in these cases it offers a more definite approach allowing maximal chances to find testicular spermatozoa.

References


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Mode of delivery after uterine metroplasty

Dear Sir,

We write in response to the recent debate in Human Reproduction, ‘Shall we operate on Müllerian defects?’ (for example Pellicer, 1997). A number of operative options are available, such as the Strassman procedure (Strassman, 1907) which is the only abdominal operation described to treat the bicornuate uterus or uterus didelphys. The success of this procedure in patients with a bicornuate uterus has been long established. The percentage of successful pregnancies increased from a pre-operative 3% to 83% in a series reported by Ayhan et al. (1992). Other studies including different types of uteri and various surgical techniques report similarly impressive results (for example Candiani et al., 1990).

Once the operation has been performed the argument moves to the preferred mode of delivery in a subsequent pregnancy. Many gynaecologists favour delivery by Caesarean section following an abdominal metroplasty, but advance the view that patients having undergone hysteroscopic resection of a uterine septum may be allowed a vaginal delivery in the absence of obstetric indications to the contrary (Jacobsen and DeCherney, 1997).

We would like to present our patient, a 39 year old para 0+5. In 1977 she underwent a first trimester suction termination of pregnancy on social grounds. In 1978 she suffered a first trimester missed abortion and in 1983 a first trimester incomplete abortion, both of which necessitated uterine evacuation. In 1984 she experienced a second trimester spontaneous abortion at 22 weeks gestation. Chromosome analysis revealed an apparently normal female karyotype. In 1985 investigations revealed the presence of a bicornuate uterus and accompanying cervical incompetence. A further pregnancy ensued that year, during which cervical encerclage was performed at 15 weeks gestation. At 16 weeks gestation, despite tocolysis, a further spontaneous abortion occurred. In 1986, a Strassman uteroplasty was performed followed by the initial insertion of an intrauterine contraceptive device which was subsequently removed, to maintain patency of the cavity.

In the present pregnancy, the patient attended for booking at 8 weeks gestation. The pregnancy was uncomplicated until 25 weeks gestation when she was admitted electively for rest and weekly corticosteroid injections. Four days after admission, spontaneous rupture of membranes occurred. The patient was apyrexial with a white cell count of 10.6 (4.0–11.0×10^9/l); a decision was taken to opt for conservative management unless an alteration in the clinical picture warranted intervention. Three days later uterine activity commenced spontaneously. Progress in labour was steady and the patient was therefore allowed to progress to a spontaneous vertex delivery. The first stage of labour lasted 6 h, the second 1 h 27 min and the third 8 min.

The baby was a girl weighing 920 g with Apgar scores of 9 at 1 min and 10 at 5 min, who was electively intubated at 5 min of age. Despite various problems of prematurity her neonatal progress was good.

Interestingly, of 71 known pregnancies in Strassman’s collected series (Strassman, 1952), 61 were delivered vaginally. There were no cases of uterine rupture during pregnancy or delivery. In the light of this and our own experience, it may be that automatic recourse to Caesarean section in patients who have undergone abdominal operative correction of Müllerian defects is not justified, and an operative delivery should be reserved for obstetric indications alone.

References


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Dear Sir,

In response to the letter from Lyall and Lunan, our opinion can be expressed as follows: first, there is controversy as to
whether an abdominal metroplasty should be terminated by vaginal delivery. Although they mention the experience of Strassmann (1952, 1956) in which the vaginal route was elected, other authors have shown an incidence of uterine rupture in 2–4% of cases (Kaser, 1981; Ayhan, 1992). Thus, we believe that the risk, although low, is sufficient to indicate a Caesarean section in cases of laparotomic metroplasty. However, the fact that metroplasties are performed today by hysteroscopy in most of the cases makes the picture quite different, and there are reports in the literature which confirm the safety of vaginal delivery after hysteroscopic metroplasty (Daly et al., 1983, 1989; Fayez, 1986; March et al., 1987; Guarinos et al., 1989; Maneschi et al., 1991; Arcaini et al., 1995; Colacurci et al., 1996; Bacsko, 1997; Donnez and Nisolle, 1997; Jacobsen and De Cherney, 1997).

The second point to be addressed is the convenience of a vaginal delivery in a pre-term fetus, which is the case presented herein. Vaginal delivery can be a factor of distress leading to intracranial haemorrhagia and/or perinatal death (Westgren, 1985; Pauerstein, 1987; Arias, 1994; Bottoms, 1995). In fact, several authors recommend delivering these fetuses by Caesarean section in order to reduce the incidence of complications from 40% to 11% (Bosche et al., 1996; Harms et al., 1996). We firmly believe that this should be the case regardless of the type of metroplasty performed.

References

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