ANDROLOGY

Performing Testicular or Epididymal Sperm Retrieval Prior to the Injection of hCG

BULENT URMAN,^{1,2} CENGİZ ALATAS,¹ SENAI AKSOY,¹ ALP NUHOGLU,¹ AYCAN SERTAC,¹ and BASAK BALABAN¹

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Purpose: Our purpose was to determine the feasibility and efficacy of performing testicular or epididymal sperm retrieval prior to the injection of human chorionic gonadotropin (hCG).

Methods: This report deals with 87 sperm-positive percutaneous epididymal sperm aspiration (PESA), percutaneous testicular sperm aspiration (PTSA), or testicular sperm extraction (TESE) cycles. All sperm retrieval procedures were performed prior to administration of hCG to the women. Retrieved spermatozoa were cultured in vitro in simple medium for approximately 40 hr prior to intracytoplasmic sperm injection.

Results: In all but one cycle in which TESE was performed for nonobstructive azoospermia, motile sperm were available for ICSI. The overall fertilization rate was 53%. Pregnancy rate per transfer and implantation rate per embryo were 41.2 and 15.7%, respectively.

Conclusions: Satisfactory fertilization and pregnancy rates can be achieved when PESA, PTSA, or TESE is performed prior to the injection of hCG followed by in vitro culture of spermatozoa approximately 40 hr before ICSI. Scheduling of testicular or epididymal sperm retrieval cases in this way appears to ease the workload on laboratory and operating room personnel. Furthermore, withholding hCG when sperm is absent may obviate the unnecessary risk of ovarian hyperstimulation when spermatozoa cannot be retrieved.

KEY WORDS: testicular sperm extraction; percutaneous sperm aspiration; human chorionic gonadotropin; ovarian hyperstimulation syndrome.

INTRODUCTION

Retrieval of spermatozoa or precursors from the testes and subsequent intracytoplasmic sperm injection (ICSI) have become an established method for treatment of azoospermia (1, 2). Testicular sperm extraction (via open biopsy or percutaneously) or micro-epididymal sperm aspiration (via microsurgery or percutaneously) is usually performed on the same day and prior to oocyte retrieval. The timing of sperm retrieval may pose several disadvantages such as inconvenient scheduling for busy IVF clinics, unnecessary risk of ovarian hyperstimulation when oocyte retrieval is canceled due to the absence of sperm, and injection of oocytes with immotile sperm when sufficient numbers of motile sperm cannot be identified in the sample. The above concerns can be addressed if sperm retrieval can be performed prior to the injection of human chorionic gonadotropin (hCG). It already has been shown that epididymal and testicular sperm can survive in culture media for up to 3 days and induction of motility appears to be beneficial in selecting the most appropriate spermatozoa for ICSI (3).

This is a preliminary report of 87 sperm-positive treatment cycles in azoospermic males in which sperm retrieval was performed prior to the scheduled hCG injection.

MATERIALS AND METHODS

Of the initially planned 134 cycles, hCG was not administered to 47 women due to unavailability of

¹ Assisted Reproductive Technology Unit, American Hospital of Istanbul, Istanbul, Turkey.

² To whom correspondence should be addressed at VKV Amerikan Hastanesi, Guzelbahce sok No. 20, Nisantasi 80200, Istanbul, Turkey.

sperm. A total of 87 cycles of testicular sperm aspiration/extraction or epididymal sperm aspiration where spermatozoa were identified is reported in this study. Percutaneous epididymal sperm aspiration (PESA) was undertaken in men with obstructive azoospermia ($n = 11$). In the case of failure to enter the epididymis or to retrieve motile sperm, percutaneous testicular sperm aspiration (PTSA) was performed ($n = 10$). In men with nonobstructive azoospermia PTSA was attempted first ($n = 13$) and TESE was performed when needle aspiration failed to produce spermatozoa ($r = 53$). TESE was performed as described by Tournaye *et al.* (1). PESA and PTSA were performed according to Craft *et al.* (4). All procedures were performed with the patient under local anesthesia on the evening of planned hCG injection. Testicular tissue samples obtained by open biopsy were placed in Falcon tubes containing 2 ml EBSS medium supplemented with human serum albumin (HSA), penicillin, and pyruvate. Tissue samples were then divided into small segments and gently crushed with microneedles in a petri dish containing the same medium. The sperm suspension thus obtained was then centrifuged for 5 min at 300g in a Falcon tube. If sperm cells were identified, the suspension was centrifuged on a two-layer Percoll gradient (50 and 90%) to free the sperm from debris and red blood cells. Both gradients were washed with HSA-supplemented EBSS and the final solution was kept in the incubator until the ICSI procedure. No special medium was used for in vitro culture of spermatozoa. Percutaneous epididymal sperm aspiration was performed using a 21-gauge butterfly needle and epididymal aspirate was examined for motile spermatozoa. When no motile sperm could be identified, a 19-gauge butterfly needle was inserted into the testes and moved up and down to sample a wide area. An artery forceps was secured across the attached microtubing set before the needle was withdrawn. The aspirate located in the tubing was washed into a Falcon tube with a small volume of medium. The presence of spermatozoa was sought under $\times 200$ magnification. Culturing of PESA or PTSA sperm was identical to that of TESE sperm.

Controlled ovarian hyperstimulation was undertaken using a long-protocol gonadotropin releasing hormone (GnRH) analogue in combination with human menopausal gonadotropin (hMG). Buserelin acetate nasal spray, 600 μ g/day (Suprecur; Hoechst AG, Frankfurt am Main, Germany), was initiated on the twenty-first day of the menstrual cycle. Following the confirmation of down regulation (estradiol, < 50 pg/ml; progesterone, < 1 ng/ml; absence of ovarian cystic structures > 10 mm in average diameter), a combination of pFSH (Metrodin, 75 I.F.; Serono, Rome, Italy) and hMG (Pergonal, 500 I.F.; Serono, Italy) was administered in an individualized fashion starting on the third day of the menstrual period. hCG (Profazi

HP, 5000 I.F., Serono, Italy), 10,000 IU, was administered for final maturation of the oocytes when the leading follicle and at least two other follicles reached a mean diameter of 20 mm. Oocyte retrieval was performed with the patient having been administered intravenous analgesia and local anesthesia. Following denudation of the oocyte-cumulus complex with hyaluronidase, all metaphase II oocytes were injected with motile spermatozoa. The ICSI procedure was performed according to Steirteghem *et al.* (5). Tetracycline, 100 mg twice a day, and methylprednisolone, 16 mg/day, were administered for 5 days from the day of oocyte retrieval. Embryo transfer was performed approximately 48 hr after ICSI. The morphologically best-looking three or four embryos were replaced. Selective assisted hatching was performed on embryos generated from women older than 35 years, women with elevated early follicular-phase follicle stimulating hormone (FSH) levels (> 10 mIU/ml), women with more than one previous implantation failure, and embryos of suboptimal (G3 and G4) quality. The luteal phase was supported with intravaginal progesterone suppositories (Utrogestan; Laboratories Besins Iscovesco, Paris, France). Clinical pregnancy was defined as the sonographic identification of the gestational sac/s.

RESULTS

Indications for the various sperm retrieval procedures are shown in Fig. 1. In 86 treatment cycles motile sperm were available for ICSI to complete the procedure. In one patient with secretory azoospermia very few sperm were obtained with TESE, and none of these appeared to be viable following in vitro culture. The TESE procedure had to be repeated on the other testicle on the morning of oocyte retrieval. In this patient ICSI was performed with fresh spermatozoa. In 19 (29%) men with nonobstructive and 14 (67%)

Fig. 1. Sperm retrieval: indications and routes. PESA, percutaneous epididymal sperm aspiration; PTSA, percutaneous testicular sperm aspiration; TESE, testicular sperm extraction.

Table I. Comparison of ICSI Cycles with Ejaculated and Testicular or Epididymal Spermatozoa

Variable	ICSI cycles	PESA, PTSA, and TESE cycles	P value
No. oocyte retrievals	399	87	
No. embryo transfers	378	85	
Mean female age	33.9 ± 5.3	30.2 ± 4.9	0.007
Day 3 FSH	7.44 ± 4.7	5.4 ± 1.9	0.000
Stimulation (days)	10.4 ± 2.3	10.4 ± 2.1	NS
Ampoules hMG	33.8 ± 7.9	34.1 ± 8.1	NS
Estradiol at hCG (pg/ml)	2304 ± 1688	2934 ± 1598	0.02
No. follicles >14mm	7.1 ± 4.9	9.8 ± 6.3	0.03
No. eggs	11.3 ± 6.0	12.8 ± 7.1	0.04
No. embryos transferred	3.2 ± 1.9	3.9 ± 1.4	NS
Embryo score	11.1 ± 5.7	13.4 ± 5.1	NS
Pregnancies per transfer	170/378 (44.9%)	35/85 (41.2%)	NS
Implantation/embryo	254/1309 (19.4%)	53/337 (15.7%)	NS
OHSS (severe)	11/399 (2.8%)	3/87 (3.4%)	NS

men with obstructive azoospermia, sperm were frozen for later use. A total of 1044 oocytes was retrieved and 553 fertilized. Fertilization rate was 53%. Embryo transfer was possible in 85 of 87 cycles. In the remaining two cycles oocytes failed to fertilize despite the injection of motile spermatozoa. All oocytes appeared to have very dark and granular cytoplasm in one couple, but there was not obvious cause in the other.

The outcome of ICSI cycles using in vitro cultured testicular or epididymal spermatozoa compared with ICSI using ejaculated spermatozoa is shown in Table I. As is evident from the data, the female partners of azoospermic males were younger, had lower early follicular-phase FSH levels, reached higher serum estradiol levels on the anticipated day of hCG, and had more follicles greater than 14 mm at the completion of controlled ovarian hyperstimulation. They also yielded significantly more oocytes to aspiration. The number of embryos transferred and the pregnancy rate per transfer did not differ between the groups. The incidence of severe ovarian hyperstimulation syndrome (OHSS) was also similar. There was no severe OHSS in the group of women who did not receive hCG due to the absence of sperm cells in their male partners.

DISCUSSION

Clinics offering assisted reproductive techniques are often very busy and sperm retrieval from azoospermic males may pose difficulty in scheduling cases due to lack of sufficient rooms in the operating theater, immediate availability of surgeons, and shortage of embryologists. Performing these procedures prior to oocyte retrieval obviates most of the above concerns.

Testicular sperm are often immotile upon extrac-

tion. It was demonstrated previously by Zhu *et al.* that incubation of sperm for periods ranging from 48 to 72 hr induces motility and assists in selecting viable sperm for injection of oocytes (3). Liu *et al.* reported on the difference in outcome of in vitro cultured testicular sperm between men with obstructive and men with nonobstructive azoospermia. These authors showed a significant increase in motility of testicular sperm retrieved from men with obstructive azoospermia that were cultured for 72–96 hr in simple medium (6). However, in men with nonobstructive azoospermia, in vitro culture appeared detrimental to sperm survival, therefore, the authors suggested retrieval and injection on the same day of oocyte retrieval. In our series only in one man with nonobstructive azoospermia did the procedure need to be repeated due to the absence of viable sperm following in vitro culture of approximately 40 hr. Larger case series may determine whether this may indeed be an important obstacle.

We observed that female partners of azoospermic men are younger when treatment with assisted reproductive techniques is instituted, as there is a shorter delay from diagnosis of the condition to referral. Our data confirm this observation, as wives of azoospermic men were significantly younger, had lower basal FSH levels, reached higher peak estradiol levels, had more preovulatory follicles available for aspiration, and yielded more oocytes. All of the above parameters are considered risk factors for OHSS. Scheduling sperm retrieval on the afternoon of planned hCG injection and withholding hCG when sperm is absent may therefore obviate the unnecessary risk of OHSS in women whose husbands are not able to produce spermatozoa.

In conclusion, retrieval of spermatozoa from azoospermic men can be performed prior to hCG injection to the woman. This protects the woman from unnecessary risk of OHSS in case spermatozoa are not available. It also may confer additional benefit in terms of inducing motility in otherwise immotile or weakly motile sperm cells. Furthermore, the scheduling of cases as such was enthusiastically supported by the laboratory personnel and also appeared to be favorable in terms of decreasing the clinicians' work load in the busy morning hours.

The best approach to the couple suffering from infertility due to nonobstructive azoospermia would be to institute treatment after noninvasively predicting the availability of sperm in the testes. This necessitates sufficiently accurate tests with a high positive and negative predictive value. Until such tests are available sperm can either be retrieved prior to controlled ovarian hyperstimulation and cryopreserved for later use (7, 8) or be retrieved on the day of oocyte pickup. The use of frozen-thawed testicular or epididymal sperm has been shown to be efficacious, especially when sufficient numbers of spermatozoa can be retrieved. Compared with ICSI using fresh spermatozoa, ICSI

using frozen-thawed spermatozoa appears to yield similar results. However, when only immotile sperm are present after thawing, testicular exploration has to be repeated. Retrieval of sperm prior to the injection of hCG appears to be a viable alternative to retrieval of sperm on the day of oocyte recovery, conferring scheduling advantages and protecting the women from OHSS in case spermatozoa are not available.

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