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Azoospermic Patients: Mosaic Pattern in Testicular Sperm Extraction

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ABSTRACT

OBJECTIVE: To study the mosaic (focal) pattern of spermatogenesis in azoospermic patients.

MATERIALS AND METHODS: We conducted a cross-sectional study between June 2003 and February 2007 that included 87 non-obstructed azoospermic patients who underwent testicular sperm extraction (TESE) mapping. The extraction of spermatozoa into a culture medium was compared with testicular histology results. Both histopathology and TESE results were reviewed independently and blindly. Positive mosaic (focal) spermatogenesis was considered in positive mapping or positive TESE with a negative histology.

RESULTS: Mosaic pattern was identified in 26.44% (23 patients) of the study sample. There was no difference in mosaic distribution between testes. There was a mosaic pattern in 10 of the 22 (22.22%) right testes tested and in 13 of the 42 (30.95%) left testes ($P = 0.071$; CI = 0.10209-0.27669). Histopathological background showed a difference in mosaic distribution. Mosaic pattern was identified in association with maturation arrest in 12 (13.79%) patients, testicular atrophy in 3 (3.45%), atrophy and maturation arrest in 1 (1.14%), Sertoli cell in 3 (3.45%), hypospermatogenesis in 2 (2.3%), and mixed histopathology in 2 (3.45%), in which atrophy, maturation arrest, and hypospermatogenesis all were identified in the same histopathological sample ($P = 0.055$; CI = 0.050-0.059).

CONCLUSION: Mosaic pattern of spermatogenesis was found in 26.44% of men with non-obstructive azoospermia. Mosaic (focal) spermatogenesis is more often identified in the histological background of maturation arrest. The chance of sperm retrieval is improved by taking biopsies from multiple sites of the testes.

KEYWORDS: TESE, Mosaic TESE, Azoospermic patients, Mosaic pattern of spermatogenesis in azoospermic patients, Mosaic pattern of spermatogenesis

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INTRODUCTION

Advanced techniques of assisted reproduction have revolutionized treatment for male infertility. In particular, the introduction of intracytoplasmic sperm injection (ICSI) in combination with in vitro fertilization (IVF) has offered, for the first time, therapy with a high chance of producing a pregnancy from an azoospermic patient [1-4]. In addition, a number of novel techniques for spermatozoa retrieval have been introduced, including microsurgical epididymal sperm aspiration (MESA), percutaneous epididymal sperm aspiration (PESA), and testicular spermatozoa extraction (TESE) [3,5-9].

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However, several reports have indicated that TESE can also be applied in cases of serious testicular damage (including multifocal atrophy of seminiferous tubules) or disturbance of spermatid differentiation [10-18].

Pathological spermatogenesis might be focal, which underlines the need for multiple TESE biopsies. Only a few studies have investigated this claim under controlled circumstances [19-22]. In our study, we looked for evidence to support the hypothesis of focal spermatogenesis in non-obstructive azoospermia.

MATERIAL AND METHODOLOGY

A cross-sectional study was conducted between June 2003 and February 2007 and included all azoospermic patients (confirmed by minimum of 2 semen analyses) presenting with primary infertility and undergoing TESE (testicular mapping). Inclusion criteria included non-obstructive azoospermic patients, while obstructive patients were excluded. The experimental protocol was approved by the local ethics committee.

Procedure

TESE was performed under local anesthesia (both spermatic and scrotal). A transverse incision was done through the scrotum and dartous until the tunica was identified. A small incision was performed to obtain testicular tissue. The specimen was then sent to the embryologist for examination. If the embryologist did not identify any sperm, the surgeon proceeded with testicular mapping. The surgeon took 3-7 testicular biopsies (mean = 5) randomly before moving to the contralateral testis. If still no sperm were found, the TESE was considered negative. If sperm were identified, more specimens were taken from the positive site for cryopreservation for further ICSI. Concomitantly, 1 specimen was sent for histopathology evaluation for all patients.

Examination of the TESE by the embryologist

The testicular tissue was immediately immersed into 1.0 mL of Earle's medium (gassed overnight; supplemented with 4 mM of sodium bicarbonate, 21 mM of HEPES, 0.47 mM of pyruvate, and 10% vol/vol synthetic serum substitute), placed in sterile tube, and transferred to the andrology laboratory of the IVF unit. It was then transferred to a sterile, 2.5-cm petri dish (Falcon 3002, Becton Dickinson and Co., Inc., Franklin Lakes, NJ) and maintained at 37°C.

Under a dissecting microscope, the seminiferous tubules were teased apart using 21-gauge needles. Using sterile slides, the contents were squeezed into the surrounding media and examined under a dissecting microscope for the presence or absence of mature sperm.

Processing for cryopreservation of testicular tissue

The tubules were transferred to a 15-mL conical tube containing 1 mL of fresh media, and the cell suspension was transferred to a separate centrifuge tube. Both tubes were incubated at 37°C for 15–30 minutes, and the supernatant of the first tube (containing tubules) was combined with the cell suspension in the second tube. The suspension was centrifuged at 500× *g* for 5 minutes, and the pellet was resuspended in 1 mL of media with 0.3% BSA. A cell count was performed, and the suspension was diluted or concentrated to 0.5–1.0 million sperm/mL. Before freezing, an aliquot was removed to assess sperm quality.

At cryopreservation, multiple aliquots of sperm were frozen whenever possible. The cell suspension was slowly diluted 1:1 with sperm freezing media supplemented with glycerol. The samples were slow cooled at a rate of –0.5°C/min to 4°C and then packaged in 1-mL cryovials (Nunc Cryotubes, Roskilde, Denmark). The vials were frozen at a rate of –10°C/min to –90°C and were stored in liquid nitrogen at –196°C.

Histopathology

Pieces of testicular biopsy were fixed immediately in Bouin's solution. Semi-thin paraffin wax sections (4 micron) were stained and examined by light microscopy at x400 magnification using standard techniques. The slides were read by 3 assessors unaware of the results of the TESE. Testicular histology was classified into hypospermatogenesis (reduction in the degree of normal spermatogenic cells), maturation arrest (an absence of the later stages of spermatogenesis), Sertoli cell only (the absence of germ cells in the seminiferous tubules), atrophy, obstructed (normal spermatogenesis), or mixed (more than 1 histopathology type).

Mosaic criteria

Both histopathology and TESE results were reviewed independently and blindly for the presence of spermatozoa. Next, results were recorded and reviewed by the investigator for the constructed mosaic (focal) spermatogenesis criteria. Positive mosaic spermatogenesis was considered if there was positive mapping (i.e. moving from a negative to a positive spot in the testis) or positive TESE with a negative histology.

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Analytic study

We used the SPSS computer program (v.15 for Windows) for data analysis. Values are presented as percentages and *P* values with 95% confidence intervals. A *P* value of < 0.05 was judged as statistically significant.

RESULTS

A total of 87 patients with mean age of 31 years and a history of primary infertility for an average of 2-13 years were included in the study. Mosaic pattern of spermatogenesis was identified in 26.44% (23) of patients. There was no difference in mosaic distribution between testes. Mosaic pattern was present in 10 of the 45 (22.22%) right testes tested and in 13 of the 42 (30.95%) left testes (*P* = 0.071; CI = 0.10209-0.27669). Histopathological background showed a difference in mosaic distribution. Mosaic pattern was identified in association with maturation arrest in 12 (13.79%) patients, testicular atrophy in 3 (3.45%), atrophy and maturation arrest in 1 (1.14%), Sertoli cell in 3 (3.45%), hypospermatogenesis in 2 (2.3%), and 2 (3.45%) patients had mixed histopathology in which

Table 1. Distribution of mosaic pattern according to the age

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Mosaic	20-29*	30-39	40-49	50-59	70-79	Total
Positive	6	15	1	1	0	23
Negative	13	41	5	4	1	64
Total	19	56	6	5	1	87

*Age in years

Table 2. Distribution of mosaic pattern between the right and left testis

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Mosaic pattern	RT testis	LT testis	Total
Positive	10	13	23
Negative	35	29	64
Total	45	42	87

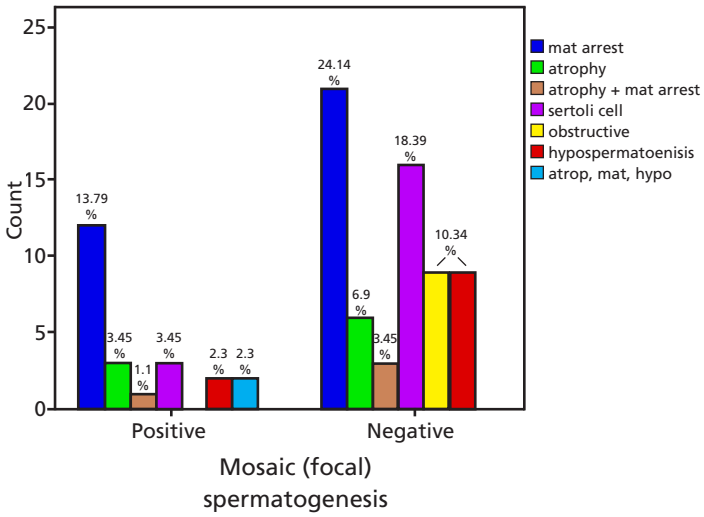

Figure 1. The distribution of positive and negative mosaic spermatogenesis according to the different testicular histopathological background

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Table 3. Distribution of mosaic spermatogenesis according to the histopathology background

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Mosaic	Maturation arrest	Atrophy	Atrophy and maturation arrest	Sertoli cell	Obstructive	Hypospermatogeneses	Mixed morphology	Total
Positive	12	3	1	3	0	2	2	23
Negative	21	6	3	16	9	9	0	64
Total	33	9	4	19	9	11	2	87

atrophy, maturation arrest, and hypospermatogenesis all were identified in the same histopathological sample (P = 0.055; CI = 0.050-0.059). The results are shown in Table 1, Table 2, Table 3, and Figure 1.

DISCUSSION

It is well established that mature testicular spermatozoa can be found in cases of non-obstructive azoospermic men [23,24]. Moreover, it has also been proven that such sperm has fertilization ability after the intracytoplasmic sperm injection (ICSI) procedure [12,25-28]. These findings made the achievement of genetic offspring possible for a population of men who were previously advised to use donor spermatozoa. As a result, testicular sperm retrieval procedures in non-obstructive azoospermic men are becoming increasingly popular.

At present, there are no means of predicting the presence of mature spermatozoa in non-obstructed azoospermic men except by performing testicular biopsy [23,24,29].

In fertile men, sperm cells are produced throughout the testis. Steinberger and Tjioe [30] and Roosen-Range [31] stated that a single large sample is representative of the entire testis. Levin [32] examined the testes of infertile men and found mixed histological patterns of germinal cell aplasia and focal spermatogenesis in 6% of patients. A similar histology of side-by-side presence of different patterns was observed in non-obstructed azoospermic men [25,26,33-36]. The incidence of such mosaic pattern of spermatogenesis has not been determined previously. However, our study showed an incidence of 26.4% for the mosaic pattern of spermatogenesis in azoospermic patients.

There was no difference in the pattern of spermatogenesis between the right and left testes (P = 0.071), but different testis histology showed an effect on the pattern of spermatogenesis. We found the maturation arrest to have the highest incidence (13.79%) of mosaic pattern in comparison to the other histology (Table 3, Figure 1).

CONCLUSION

Mosaic pattern of spermatogenesis was found in 26.44% of men with non-obstructive azoospermia. Mosaic (focal) spermatogenesis is most often identified in the histological background of maturation arrest. The chance of sperm retrieval is improved by taking biopsies from multiple sites of the testes.

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