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ANDROLOGY

A Comparative Study of Three Techniques for the Analysis of Sperm Recovery: Touch-Print Cytology, Wet Preparation, and Testicular Histopathology

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Purpose: *The aim of this study is to evaluate the efficacy of simultaneous testicular touch-print cytology, testicular histopathology, and wet preparation in nonobstructive azoospermic (NOA) males.*

Methods: *Three hundred and sixty-three males with NOA underwent a multiple testicular sampling prior to ICSI for histopathologic evaluation, diagnostic testicular sperm extraction, and simultaneous touch-print cytology to evaluate sperm presence or absence. A total of 979 testicular samples were taken.*

Results: *Sperm recovery was achieved in 106 cases (29.2%). Patients with hypospermatogenesis and focal spermatogenesis needed 2.8 and 5.9 biopsies, respectively, to retrieve spermatozoa, while in patients with germ cell aplasia and maturation arrest, even after eight to nine samples no spermatozoa were recovered. Neither the FSH levels nor the testicular volume was found to be significant in the prediction of sperm recovery. If only a single testis was to be biopsied, 25% of the cases with sperm recovery would have been missed. The combination of touch-print cytology with histopathology and wet preparation increased the accuracy of spermatozoa identification.*

Conclusion: *Touch-print cytology was found to be more predictive than wet preparation in the diagnosis of spermatogenesis; moreover, it was found to be a quick and easy technique providing an accurate diagnosis in prediction of sperm recovery.*

KEY WORDS: nonobstructive azoospermia; testicular biopsy; touch-print cytology; wet preparation.

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INTRODUCTION

Multiple testicular sampling is a widely accepted practice based on multifocal spermatogenesis in nonobstructive azoospermic (NOA) males (1–4). Testicular biopsy is a simple, minimally invasive procedure with little attendant morbidity. When multiple testicular biopsies are performed, they provide important information on the diagnosis and prognosis of treatment in azoospermic males.

Histopathological evaluation of testicular biopsy specimen plays a significant role in diagnosis of the severity of defective spermatogenesis. However, it is difficult to distinguish late spermatids from mature sperm with histopathologic evaluation of testicular tissue prepared by routine hematoxylin and eosin staining. By histopathologic evaluation, the whole structure of a sperm cell cannot always be identified since the sperm head and tail may not be distinguishable on the same section. Testicular sperm extraction (TESE) in combination with touch-print cytology and histopathology may provide a more

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accurate diagnosis in the detection of sperm presence or absence (5–8). Diagnostic accuracy of testicular biopsy may be significantly improved when touch-print cytology, histopathology, and wet preparation are combined since spermatozoa and round cells can be clearly identified, particularly with inverted microscope equipped with phase contrast attachments (6,9).

The aim of this study is to compare three different techniques, testicular histopathology, wet preparation, and touch-print cytology, in terms of diagnostic accuracy for the evaluation of NOA patients.

MATERIALS AND METHODS

This study was performed in Sevgi Hospital ART and Reproductive Endocrinology Unit between 1997 and 1999. Three hundred and sixty-three males who underwent diagnostic multiple testicular biopsies were evaluated and subsequently diagnosed as NOA. Simultaneous diagnostic TESE and touch-print cytology were performed. Histopathology, touch-print cytology, and wet preparation were studied in terms of sperm recovery in NOA males. The mean ages of female and male partners were 32.2 ± 0.36 and 35.8 ± 0.27, respectively.

The mean duration of infertility was 9.26 ± 0.25 years. The mean seminal volume was 3.27 ± 0.89. When spermatozoa were recovered with testicular tissue extraction, they were cryopreserved in as many aliquots as possible for later use in ICSI. Multiple testicular tissue samples (3–12 samples) were biopsied. Testicular spermatozoa could be successfully recovered by TESE in 106 out of 363 patients (29.2%). In 30.1% of the cases, motile testicular spermatozoa were observed.

Testicular histology was classified into hypospermatogenesis (reduction in the degree of normal spermatogenetic cells), maturation arrest (MA; an absence of later stages of spermatogenesis), Sertoli cell only (the absence of germ cells in the seminiferous tubules), and focal spermatogenesis (small areas of apparently normal spermatogenesis). Testicular histology was scored on a scale of 1–10 according to the Johnsen's modified scoring criteria (10). In each slide, 100–200 tubules were examined. Each tubular spermatogenetic activity was evaluated according to the percentage of each histomorphological findings. Seminiferous tubules are scored on a scale of 1–10, with tubules having complete inactivity, scored as 1 and those with maximum activity of at least five or more spermatozoa in the lumen were scored as 10.

Biopsy specimens were taken from the testicles under local anesthesia through a 2- to 3-cm scrotal incision. The extracted testicular pieces were fixed immediately in Bouin's solution. Semithin paraffin was sections were stained and examined under a light microscope at $\times 400$ magnification using standard techniques. Histological examination was combined with an immediate postsurgical test (wet preparation). Testicular tissue samples were placed in a Falcon tube (Becton Dickinson, NJ) containing 1 mL of HEPES-buffered Earle's medium (Gibco, Eggenstein, Germany). The testicular tissue, which was progressively divided into small segments, was gently crushed between forceps and microneedle in a Petri dish containing HEPES-buffered medium to obtain a suspension of spermatozoa. The suspension of spermatozoa was then transferred into a Falcon tube and rotated for 50–60s with a vortex. Isolation of a single spermatozoon was considered satisfactory for ICSI. If a sufficient number of spermatozoa were obtained cryopreservation was performed.

Following testicular biopsy, touch-print cytology was performed by rapidly touching the excised testicular tissue 8–10 times onto a microscope slide. For gentle and proper specimen handling, involving touching rather than smearing after an open testicular biopsy, the biopsy specimen is held with forceps and touched many times onto a microscope slide. The slide is then immediately sprayed with cytological fixative before staining so as to avoid possible damage to cell structure when it dries (5). The slide was processed with routine hematoxylin and eosin staining and diagnosis was performed within 5 min.

Figure 1 demonstrates the histopathological features of germ cell aplasia (GCA) with the absence of germ cells in the seminiferous tubules. Cumulus of Sertoli cells is evident in touch-print cytology.

The data were analyzed using Statistical Package for Social Services (SPSS, Chicago, IL). The significance of associations between different variables was tested by chi square test, Student's *t* test, two independent proportion test, Fisher's exact test, and Mann–Whitney and Kruskal–Wallis tests where appropriate. The level of statistical significance was defined as $p \leq .05$. The sensitivity and specificity of touch-print cytology and wet preparation were calculated using regression curve analysis.

RESULTS

A total of 363 patients had diagnostic testicular biopsy and 57% were found to have GCA.

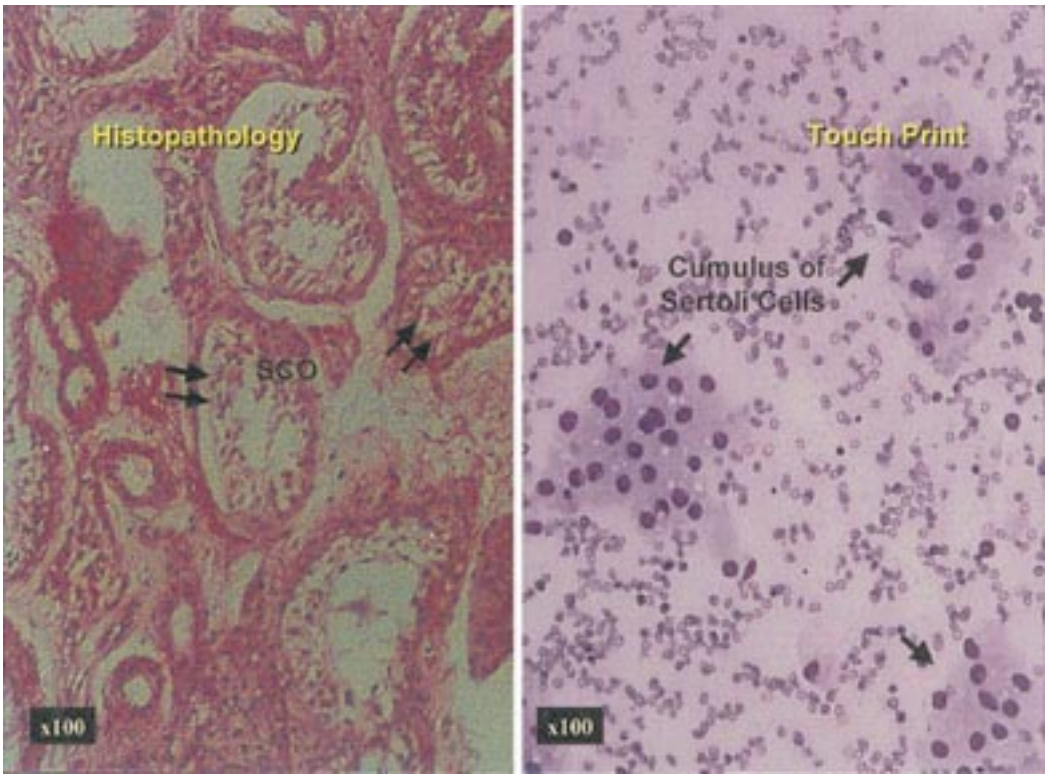


Fig. 1. Germ cell aplasia with an absence of germ cells in the seminiferous tubules (left). Cumulus of Sertoli cells is evident in touch-print cytology (right). SCO: Sertoli cell only.

Thirty-seven percent of patients had focal spermatogenesis. Eleven percent of patients had complete MA and mixed histopathology, while only 6% had hypospermatogenesis.

Patients with hypospermatogenesis needed only 2.8 biopsies in order to retrieve spermatozoa, while in patients with GCA and MA even after 8 or 9 biopsies, no spermatozoa were retrieved. Figure 2 exhibits the comparison of mean number of testicular biopsies needed to retrieve spermatozoa, in relation to testicular histopathology. In patients with Complete Germ Cell Aplasia (CGCA), a mean

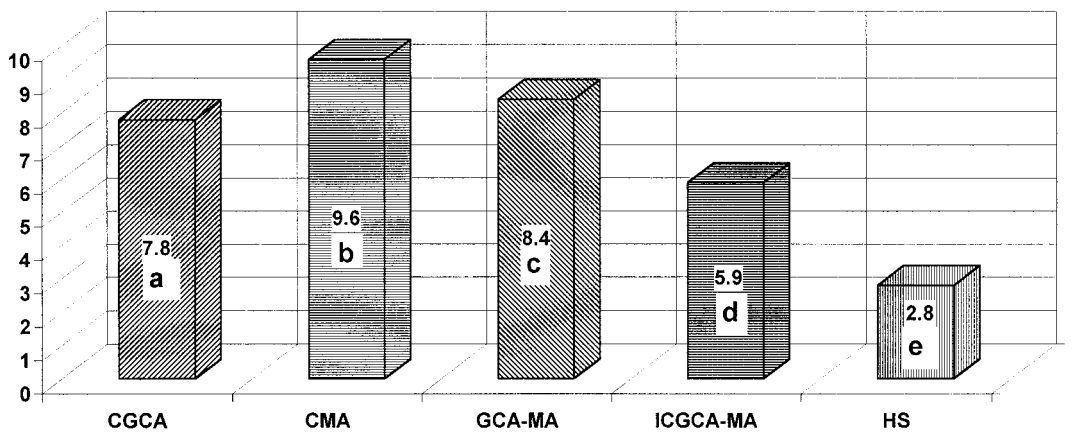


Fig. 2. Distribution of mean numbers of total testicular biopsies in relation to testicular histopathology. CGCA: Complete Germ Cell Aplasia; CMA: Complete Maturation Arrest; GCA+MA: Mixed Histopathology; ICGCA+MA: Focal Spermatogenesis; and HS: Hypospermatogenesis (Mann–Whitney U test: $p < .05$ ("abc" and "de")).

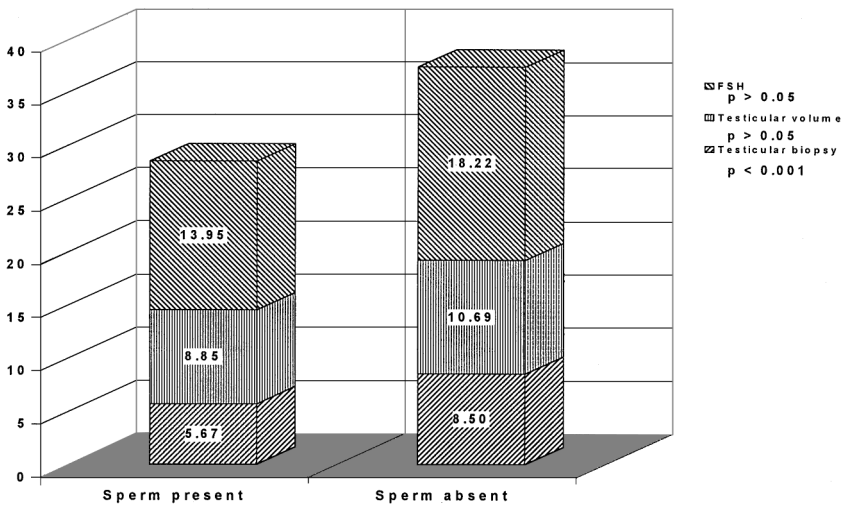


Fig. 3. Rates of mean FSH levels, testicular volumes, and total number of testicular biopsies in relation to sperm presence and absence.

number of 7.8 biopsies; in Complete Maturation Arrest (CMA) group, 9.6 biopsies; and in patients with mixed histopathology (CGCA–MA), 8.4 biopsies were taken. However, in patients with focal spermatogenesis 5.9 pieces were needed in order to retrieve spermatozoa. This number was only 2.8 in patients with hypospermatogenesis ($p < .05$).

There was no statistical significant difference between the FSH levels in relation to the testicular histopathology. The mean value of FSH was 18.2 ± 1.9 for the group in which spermatozoa could be recovered. On the other hand, mean FSH level was 13.9 ± 1.07 in cases with no spermatozoa and the difference was not statistically significant ($p > .05$). An interesting point was that the highest FSH levels were found in the hypospermatogenesis group.

In the wet preparation where spermatozoa were found, the mean number of testicular biopsies was found to be 5.6. However, the mean number of testicular samples to diagnose the absence of spermatozoa was 8.5. There was a statistically significant difference between the number of testicular biopsies in sperm presence and absence group ($p < .001$). Neither the FSH values nor the testicular volume was found to be significant in the prediction of sperm presence and absence ($p > .05$) (Fig. 3).

Patients with focal spermatogenesis or hypospermatogenesis revealed more spermatozoa than those with MA and GCA patterns. Only 6.6% sperm recovery rate was achieved in patients with CGCA; this rate was found to be 4% for CMA and 6.2% for mixed histopathology (GCA–MA) group.

The histological pattern that was highly associated with successful sperm retrieval was hypospermatogenesis (100%). In focal spermatogenesis group, sperm was recovered in 56% of cases with incomplete maturation arrest (ICMA) and in 48.3% of cases with incomplete germ cell aplasia (ICGCA) (Fig. 4). The rate of hypospermatogenesis was categorized into three groups. The first group had 5% or lower hypospermatogenesis. The second group had 5–10% and the third group had 10% and higher hypospermatogenesis. Highest sperm recovery rate was achieved in the third group.

Spermatozoa were recovered in only 13 out of 979 testicular biopsies and sperm recovery rate was found to be 1.3% in patients with CGCA and MA. Higher number of biopsies were necessary in CGCA and MA groups for sperm recovery (Fig. 5).

When 2% hypospermatogenesis is set as the cut-off point of the ROC curve for sperm recovery, a higher sensitivity and specificity of touch-print cytology were found when compared to those of wet preparation. The negative and positive predictive values of touch-print cytology were also found to be higher than those of wet preparation ($p < .0001$) (Fig. 6). Also in 13 patients out of 363 (3.5%) spermatozoa were observed in the wet preparation but not in the histological examination.

DISCUSSION

In view of focal nature of spermatogenesis in males with defective sperm production, the diagnostic value

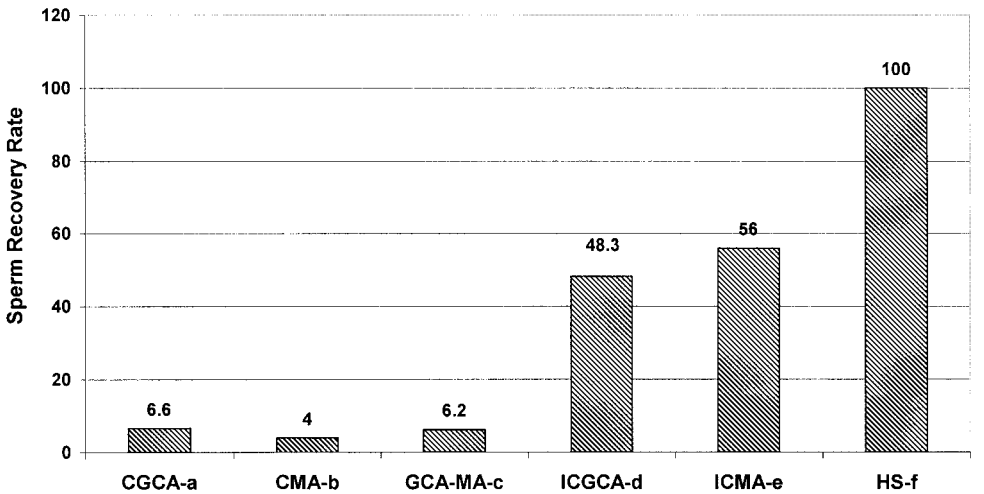


Fig. 4. Rate of sperm recovery in relation to testicular histopathology [Mann–Whitney U test: $p < .001$ ("abc" and "def")].

of a single testicular sample to predict the presence of spermatozoa in the testis is very limited. It has been shown that testicles may have islands of normal spermatogenesis surrounded by tubules characterized by arrest or absence of spermatogenetic cells (1,2,11,12).

Testicular biopsy, cytological smear, and intraoperative wet preparations are the techniques used for the detection of spermatozoa in patients with NOA. Although histological evaluation and wet preparation during diagnostic TESE are the two common procedures for diagnostic work-up, combination with touch-print cytology may enhance the efficacy of diagnosis as the whole structure of spermatozoa can be differentiated better than that by histopathology alone.

Serum FSH level, testicular volume, age, and prior history of negative or positive biopsies do not determine sperm presence or absence. In our study, highest FSH levels were found in hypospermatogenesis group with 100% sperm presence in wet preparation. Elevated FSH level does not always indicate a damaged epithelium. However it may also reflect a compensatory adaptation to partial damage resulting in subnormal sperm production (2). Neither the FSH

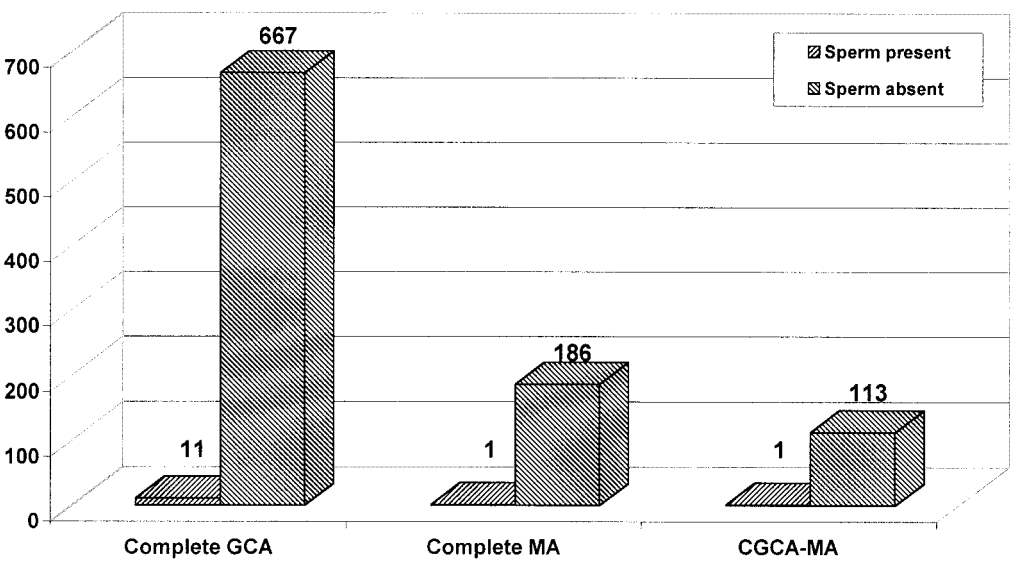
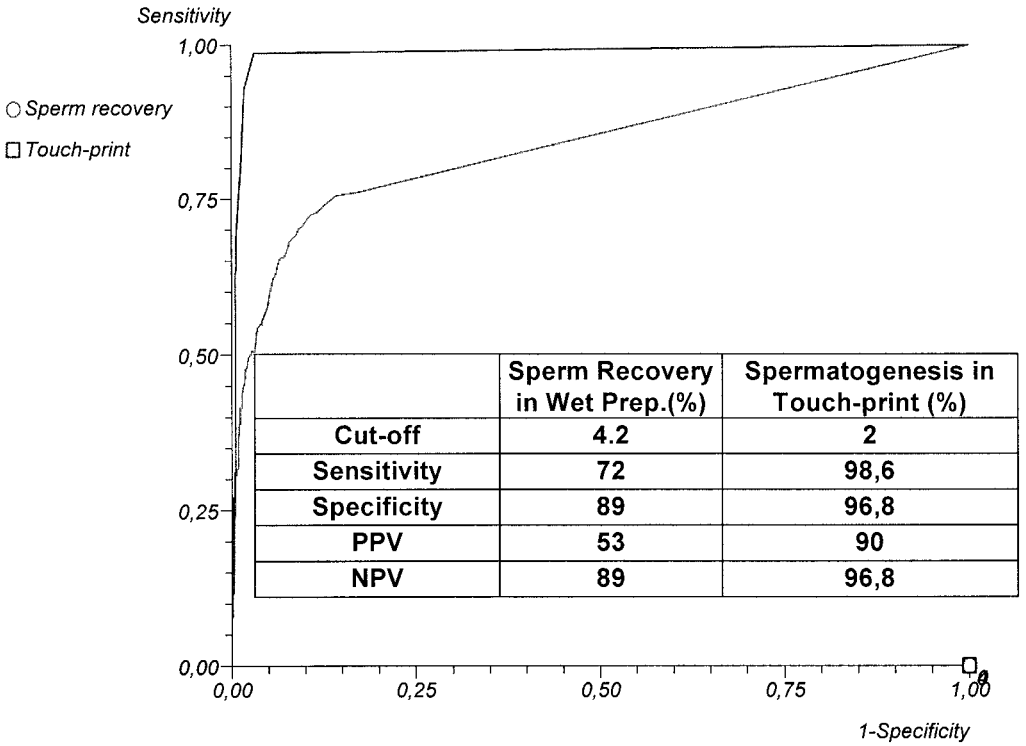


Fig. 5. Sperm recovery in comparison to biopsied testicular tissues.



	Sperm Recovery in Wet Prep.(%)	Spermatogenesis in Touch-print (%)
Cut-off	4.2	2
Sensitivity	72	98,6
Specificity	89	96,8
PPV	53	90
NPV	89	96,8

Fig. 6. ROC for sperm recovery and touch-print cytology.

levels nor the testicular volume was found to be significant in prediction of sperm recovery.

The sensitivity and specificity of touch-print cytology were higher when compared to those of wet preperation particularly when histopathology revealed low levels of hypospermatogenesis. Furthermore, negative and positive predictive values of touch-print were also higher than those of wet preparation.

As previously demonstrated by Kim *et al.*, the use of testicular touch-print cytology improves diagnostic power when performed in combination with routine histological examination of testicular tissue (13). As the whole structure of spermatozoa is often difficult to identify on testis biopsy, differentiation between MA at the late spermatid stage can be difficult. Lin *et al.* (8) described the technique of quantitative image analysis of testicular biopsy touch-preparation imprints in the evaluation of infertile men. The main advantage over the standard system of interpretation is that this method effectively provides quantification and differentiation among haploid (spermatozoa and spermatids), diploid, and tetraploid cells, based on DNA content, cell stage, and area. They concluded that performing image analysis on touch imprints demonstrates results comparable with those of analysis of paraffin-embedded sections. Spermatozoa may not be recovered in wet preparation although it may be observed during histopathological evaluation as spermatozoa may be found in a small amount of tubules. However in our study spermatozoa were observed in 13 cases of wet preparation but not at the histology (3.5%).

In conclusion a higher number of testicular biopsy is needed to decide about sperm absence in cases with CGCA and CMA while less biopsies are necessary to recover spermatozoa in cases with focal spermatogenesis and hypospermatogenesis ($p < .05$). Furthermore, touch-print cytology is more predictive than wet preparation at 2% level of hypospermatogenesis with histopathology. When histopathology represents 2% spermatogenesis, touch-print cytology is more predictive for sperm presence than wet preparation ($p < .0001$).

REFERENCES

1. Tournaye H, Staessen C, Liebaers I, Van Asche E, Devroey P, Bonduelle M, Van Steirteghem A: Testicular sperm recovery in nine 47, XXY Klinefelter patients. Hum Reprod 1996;11:1644–1649

2. Silber SJ, van Steirteghem A, Liu J, Tournaye H, Devroey P: Normal pregnancies resulting from testicular sperm extraction and intracytoplasmic sperm injection for azoospermia due to maturation arrest. Fertil Steril 1996;66:110–117
3. Kahraman S, Özgür S, Alataş C, Aksoy S, Balaban B, Evrenkaya T, Nughoglu A, Tasdemir M, Biberoglu K, Schoysman R, Vanderzwalmen P, Nijs M: High implantation and pregnancy rates with testicular sperm extraction and intracytoplasmic sperm injection in obstructive and non-obstructive azoospermia. Hum Reprod 1996;11:673–676
4. Yoshida A, Miura K, Shirai M: Evaluation of seminiferous tubule scores obtained through testicular biopsy examinations of non-obstructive azoospermic men. Fertil Steril 1997;68:514–518
5. Kim ED, Gilbaugh JH, Patel VR, *et al.*: Testis biopsies frequently demonstrate sperm in men with azoospermia and significantly elevated follicle-stimulating hormone levels. Urology 1997;157:144–146
6. Belker AM, Sherins RJ, Dennison-Lagos L: Improved simple and rapid staining method for intraoperative examination of testicular biopsy touch imprints [abstract]. Fertil Steril 1995;128(Suppl): 074
7. Kessaris DN, Wasserman P, Mellinger BC: Histopathological and cytopathological correlations of percutaneous testis biopsy and open testis biopsy in infertile men. J Urol 1995;153: 1151
8. Lin WW, Abrams J, Lipshultz LI, Kim ED: Image analysis assessment of testicular touch preparation cytologies effectively quantifies human spermatogenesis. J Urol 1998;160:1334–1336
9. Coburn M, Wheeler TM: Testicular biopsy in male infertility evaluation. *In* Infertility in Male, 2nd edn, LI Lipshultz, SS Howards (eds), St Luis, Mosby Year Book, 1991; pp 223–253
10. Johnsen SG: Testicular biopsy score count-a method for registration of spermiogenesis in human testes: Normal values and the results in 335 hypogonadal males. Hormones 1970;1:2
11. Devroey P, Liu J, Nagy ZP, Goossens A, Tournaye H, Camus M, Van Steirteghem A, Silber S: Pregnancies after testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermia. Hum Reprod 1995;10:1457–1460
12. Gottschalk-Sabag S, Weiss DB, Folb-Zacharow N, Zukerman Z: Is one testicular specimen sufficient for quantitative evaluation of spermatogenesis? Fertil Steril 1995;64:399–402
13. Kim ED, Greer JA, Abrams J, Lipshultz LI: Testicular touch preparation cytology. J Urol 1996;156:1412–1414