

## ORIGINAL ARTICLE

**Compare Open Testicular Biopsy and Testicular Fine Needle Aspiration (TEFNA) for Spermatogenesis in terms of Johnsen's Scoring in patients with Suspicion of Obstructive Azoospermia**AMANULLAH BHUTTO<sup>1</sup>, TUFAIL HUSSAIN TAHIR<sup>2</sup>, RUKHSANA SABOOR<sup>3</sup>, A SABOOR SOOMRO<sup>4</sup>, MUHAMMAD RIZWAN<sup>5</sup>, MUHAMMAD YOUNUS JAMAL SIDDIQI<sup>6</sup><sup>1</sup>Assistant Professor, Pathology Department, Ghulam Mohammad Mahar Medical College Sukkur<sup>2</sup>Associate Professor Urology Poonch Medical College, Rawalakot Azad Kashmir<sup>3</sup>Assistant Professor, Pathology Department GMMMC Sukkur<sup>4</sup>Associate Professor, SIUT Sukkur<sup>5</sup>Associate Professor, Pathology Department<sup>6</sup>Assistant Professor, Baqai Institute of Hematology.Correspondence to: Muhammad Younus Jamal Siddiqi, Email: [younus.jamal@baqai.edu.pk](mailto:younus.jamal@baqai.edu.pk)**ABSTRACT****Introduction:** Testicular biopsy is an invasive diagnostic tool to evaluate spermatogenesis and has to be performed only following strict criteria.**Objective:** To compare open testicular biopsy and testicular fine needle aspiration (TEFNA) for spermatogenesis in terms of Johnsen's scoring in patients with suspicion of obstructive azoospermia.**Materials and Methods:** This comparative study was conducted in Pathology department, Ghulam Mohammad Mahar Medical college, Sukkur during 2021 to 2022. Detailed history was taken and physical examination was carried out including examination of external genitalia. Amongst them, men with normal testicular size and bilaterally palpable vas deference were investigated further regarding their hormone profile (FSH, LH, and Testosterone) and scrotal color doppler ultrasound (CDUS). Procedure was performed under local anesthesia (Spermatic cord block).**Results:** The mean age of patients in our study was 38.9±6.4 year with minimum age as 28 and maximum as 50 year. In this study, ages were categorized in two groups; one is 20-35 years age group and second is 36-50 years age group. There were 14(35.0%) patients in 20-35 years age group, while 26(65.0%) in 36-50 years age group.**Practical implication:** This article will help to improve the technique for confirmation of presence of mature sperms.**Conclusion:** TEFNA is more reliable and minimal invasive technique for confirmation of presence mature sperms in men with obstructive azoospermia and is associated with minimal complications.**Keywords:** TEFNA, Sperms, Azoospermia, Complications**INTRODUCTION**

Testicular biopsy is an invasive diagnostic tool to evaluate spermatogenesis and has to be performed only following strict criteria. It completes history taking, physical examination, scrotal ultrasonography, ejaculate and hormonal analysis<sup>1</sup> It is indicated in cases of azoospermia, when obstruction of the genital tract is suspected because of normal testicular volume, normal follicle-stimulating hormone (FSH) levels, and low levels of epididymal ([-glucosidase, L-carnitine) or seminal vesicle (fructose) markers<sup>2</sup>.

Azoospermia, the lack of spermatozoa in the ejaculate, is the most common finding in infertile but otherwise healthy male dogs and represents an increasing reproductive health issue in men, too. The diagnosis can be further classified as non-obstructive azoospermia and obstructive azoospermia due to an obstruction of the deferent ducts<sup>3</sup>. Although non-obstructive azoospermia comprises more than half of azoospermic cases in men and is a common cause of infertility in the male dog, knowledge of the underlying etiology and pathophysiology is still strongly limited, and much uncertainty exists about the true incidence and possible treatment options<sup>4</sup>.

Infertility is a global public health issue according to the World Health Organization (Geneva, Switzerland) that affects not less than 186 million people worldwide with male infertility contributing to more than half of all cases of global childlessness<sup>5</sup>. Besides, there is sufficient evidence that male fertility or reproductive health has significantly declined over the last 40 to 60 years. Similarly to humans, infertility in dogs is a common and increasing problem. Evaluation of the predominant causes showed similar results for both species<sup>6</sup>.

Infertility is a common clinical problem that affects approximately 8% of the male population in reproductive age<sup>1</sup>. It is defined as the failure to conceive after one year of regular intercourse without contraception, and traditionally, investigation starts at this point. However, it is recommended that investigation is initiated earlier whenever risk factors are present since longer infertility duration relates to smaller chances of success regardless

of the treatment strategy adopted<sup>2-3</sup>. Determining the couple's fertility potential is a complex process that involves both male and female partners. The cause of infertility may or not be identified and can involve one or both partners. In patients of non-obstructive azoospermia, open testicular biopsy and micro-dissection testicular sperm aspiration (m-TESE) were compared for sperm retrieval. Two procedures were done in same patient simultaneously. M-TESE was found superior over testicular biopsy and no correlation was found between FSH level and sperm retrieval, which were compared additionally in the study<sup>7</sup>.

**Objective:** To compare open testicular biopsy and testicular fine needle aspiration (TEFNA) for spermatogenesis in terms of Johnsen's scoring in patients with suspicion of obstructive azoospermia.

**MATERIALS AND METHODS**

This comparative study was conducted in Pathology department, Ghulam Mohammad Mahar Medical college, Sukkur during 2021 to 2022.

**Inclusion criteria:**

- Male Gender with age > 20 years.
- Primary infertility and suspicion of obstructive azoospermia.

**Exclusion criteria:**

- Abnormal FSH, LH, Testosterone.
- Decreased testicular size on Doppler ultrasound.

**DATA COLLECTION PROCEDURES:**

Johnsen's scoring for spermatogenesis: (Leme et al., 2018)

10	Complete spermatogenesis with many spermatozoa, germinal epithelium organized in a regular thickness leaving an open lumen.
9	Many spermatozoa present but germinal epithelium shows marked sloughing or obliteration of lumen.
8	Only few spermatozoa (<5-10) present in section.
7	No spermatozoa, but many spermatids present.
6	No spermatozoa but only few spermatids (<5-10) present.
5	No spermatozoa, no spermatids Many spermatocytes present.
4	Only few spermatocytes (<5) and no spermatids and no

	spermatozoa present.
3	Spermatogonia are the only germ cell present.
2	No germ cells but Sertoli cells are present.
1	No cells in tubular section.

**Data collection:** Detailed history was taken and physical examination was carried out including examination of external genitalia. Amongst them, men with normal testicular size and bilaterally palpable vas deference were investigated further regarding their hormone profile (FSH, LH, and Testosterone) and scrotal color doppler ultrasound (CDUS). Procedure was performed under local anesthesia (Spermatic cord block). Spermatic cord was palpated and immobilized in the left hand between thumb and index finger. Needle of disposable syringe filled with 2% injection lignocaine was inserted vertically in the spermatic cord. About 3ml of lignocaine was injected in the spermatic cord. Additional 2 ml was injected by tilting syringe obliquely to spread the anesthetic effect thoroughly in spermatic cord. Additionally 1 ml of 2% lignocaine was applied at the site of incision of open testicular biopsy.

For testicular fine needle aspiration cytology (TEFNA), three specimens of seminiferous tubules were collected from upper, middle and lower parts of testis. First specimen was obtained through the biopsy incision with the 21 Fr butterfly needle which was attached with 20ml disposable syringe filled with media (sperm wash media with hepes buffer) and tubing of the butterfly needle was rinsed with media.

The data were entered and analyzed using SPSS v20.0. Comparison was made between Johnsen's scoring of open testicular biopsy tissue and tissue obtained by TEFNA.

**RESULTS**

The mean age of patients in our study was 38.9±6.4 year with minimum age as 28 and maximum as 50 year. In this study, ages were categorized in two groups; one is 20-35 years age group and second is 36-50 years age group. There were 14(35.0%) patients in 20-35 years age group, while 26(65.0%) in 36-50 years age group.

When Johnsen score was compared in both techniques, difference was found statistically significant. In open biopsy, the mean value of Johnsen score was 7.7±2.8 with minimum value as 2 and maximum as 10. Whereas in TEFNA, the mean value of Johnsen score was 9.9±0.95 with minimum value as 4 and maximum value as 10. Only one (2.5%) patient had wound infection, healed with antibiotics given for five days and one had scrotal swelling, which settled with scrotal elevation.

Table-1: Frequency distribution of age groups

Age groups	Frequency	Percent
20-35 years	14	35.0
36-50 years	26	65.0
Total	40	100.0

Table-2: Frequency distribution of wound infection

Wound Infection	Frequency	Percent
Yes	1	2.5
No	39	97.5
Total	40	100.0

Table-3: Frequency distribution of scrotal swelling

Scrotal Swelling	Frequency	Percent
Yes	1	2.5
No	39	97.5
Total	40	100.0

Table-4: Frequency distribution of spermatogenesis by open biopsy

Spermatogenesis by open biopsy	Frequency	Percent
Yes	33	82.5
No	7	17.5
Total	40	100.0

Table-5: Frequency distribution of spermatogenesis by TEFNA

spermatogenesis by TEFNA	Frequency	Percent
Yes	39	97.5
No	1	2.5
Total	40	100.0

Table-6: Presence of spermatogenesis by open biopsy vs. TEFNA

Spermatogenesis by TEFNA	Spermatogenesis by open biopsy		Total
	Yes	No	
Yes	33	6	39
No	0	1	1
Total	33	7	40

**DISCUSSION**

This is a modern study in diagnostic work up of male infertility, which maximize the chances of detection of spermatogenesis in suspected cases of obstructive azoospermia. Very limited research has been done in Pakistan on azoospermia minimal invasive diagnostic work up<sup>8</sup>.

In our clinical set-up, open testicular biopsy has remained the cornerstone in the diagnosis of male infertility for decades. Testicular FNAC has picked up in recent years following (Jarvis et al., 2018, Pintus et al., 2014) who characterized different cell types in cytological smears. Moreover TEFNA demonstrated superior accuracy of cytological diagnosis with histological categories in non-obstructive azoospermia. Additionally, FNAC is a minimally invasive technique in diagnosis and management of azoospermia<sup>9</sup>.

In current era, micro-assisted fertilization techniques are of great help for infertile couples as nowadays the only requirement in these techniques is a viable sperm and ovum. Neither quantity nor degree of motility is essential for assisted-reproductive technique<sup>10</sup>. Therefore in cytological smears a report of presence or absence of mature sperm is adequate

The results of this study of comparison between testicular FNAC and OTB in male infertility is in concordant with various studies conducted in the past and is compared with some of these studies here<sup>11</sup>. The mean age of patients in various studies are as, 27 years, 34 years, 36 years, 27 years and in our study 35.90 years<sup>12</sup>.

Dajani et al in 1998 had studied the use of testicular FNAC by grading the cytological smears in 1000 infertile men and found the common grade to be grade C which indicates SCOS. Our study showed that grade B is the commonest grade<sup>13</sup>.

(Mourad et al., 2005a) had studied the patterns of testicular histopathology in male infertility and ranked the testicular biopsies according to the Johnsen scoring system. In (Mourad et al., 2005a) the predominant Johnsen score was 2 indicating SCOS. Testicular fine-needle aspiration of mature sperm features superior capability in detecting active sites of spermatogenesis in deeper parts of testicular tissue<sup>13</sup>. There are, however, apparent controversies in the literature regarding the SRR by TESA in comparison to conventional methods such as TESE. (Arango et al., 2006) conducted the first well-designed study regarding the SRR by TESA versus TESE in a cohort of 37 men with NOA. Our study also highlighted superior spermatogenesis detection with TAFNA as compared to OTB on basis of Johnsen scoring<sup>14</sup>.

Nevertheless, some observational studies have shown comparable SRR by these two methods. (Mourad et al., 2005b) performed 111 TESA cycles with a mean of 15 punctures for aspiration in each testis on 85 azoospermic men and detected mature testicular spermatozoa in 65 (58.5%) cycles from 50 (58.8%) patients, our method of needle aspiration of whole testicular tubule, detect more mature sperms for maturity analysis<sup>15</sup>.

A large-scale study reported mature sperm in 63% of TESA procedures using a 21-G needle (Mehrotra and Singh, 2000). It was found here that TESA, on the whole, resulted in significantly lower SRR compared with TESE. The SRR, however, was comparable between TESA and TESE in subgroups of patients with serum FSH <15 IU/l and/or those with testicular histology of hypospermatogenesis<sup>16</sup>.

## CONCLUSION

TEFNA is more reliable and minimal invasive technique for confirmation of presence mature sperms in men with obstructive azoospermia and is associated with minimal complications.

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