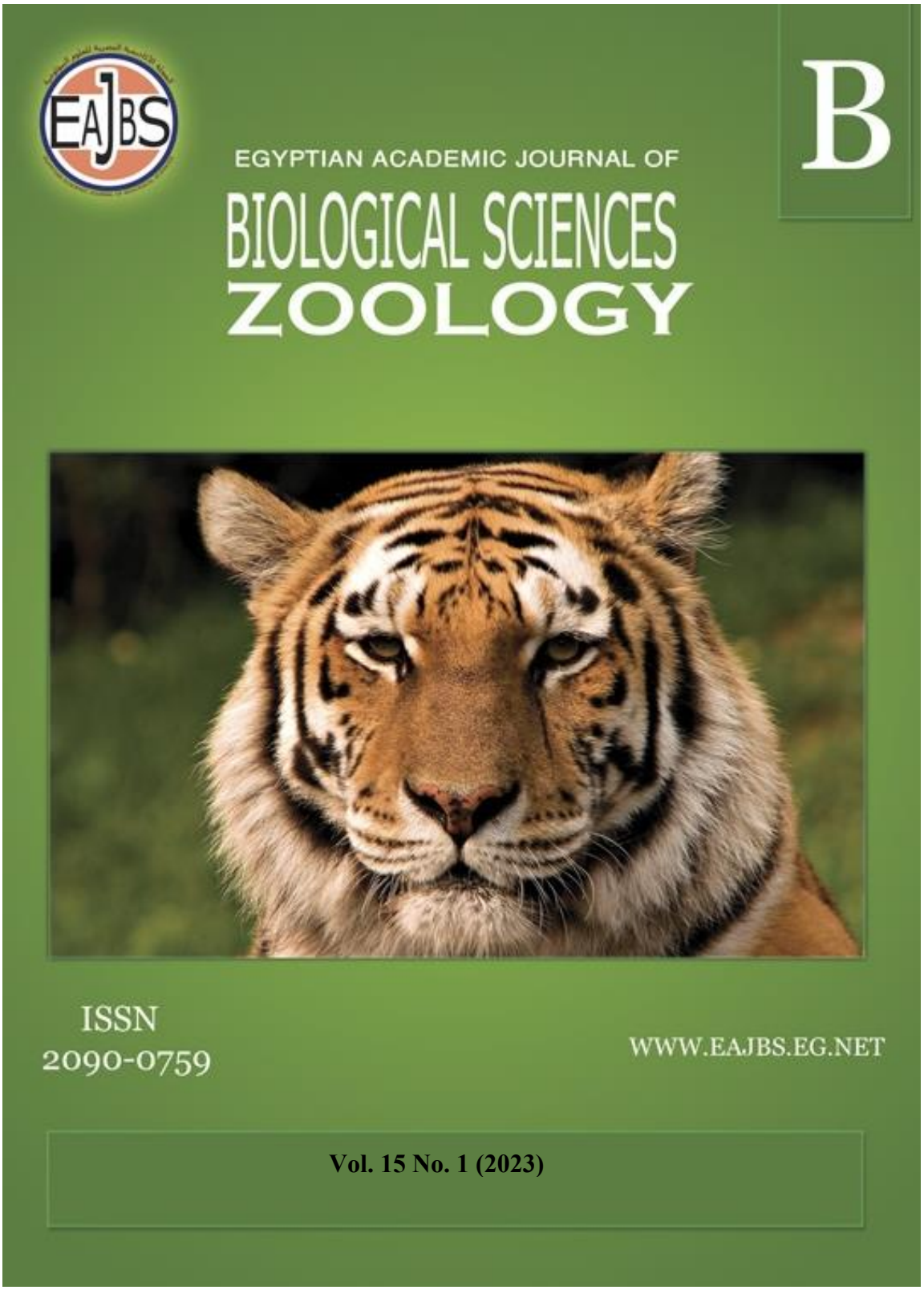

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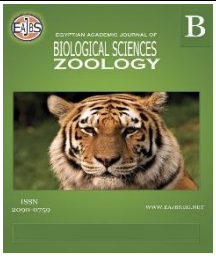

Comparison between Fresh and Histopathological Examination of Testicular Biopsy in NOA Patients

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The present work investigates the outcome of Fresh Testicular Biopsy in Comparison to Histopathological Examination in NOA Patients. In the current study, there were nine discordant instances (16.9%) between the andrology lab and histology. Histopathology characterised these cases as hypospermatogenesis, but andrology reported one case of SCO, three cases of spermatocyte, and five cases of late spermatid. To our knowledge, no study done before to examine the biopsy from NOA patients who have negative sperm retrieval in a fresh examination on the side of histopathological examination. Our intention of this study is to compare and evaluate the difference between both fresh and histopathology examinations in order to test our Andro lab results in such patients. We found in our series that (16.9%) of the patients have sperms in histopathology examination in whom we couldn't retrieve sperms in Andro lab. These results may guide us towards Auditing and improvement of a fresh examination of testicular biopsy.

INTRODUCTION

The lack of pregnancy following 12 months of regular, unprotected intercourse is the conventional clinical definition of infertility. However, it is commonly known that many couples conceive after more than a year without therapy (Collins *et al.*, 1983). Sperm functions are critical for healthy fertilization; male infertility is most often caused by sperm male function. More significantly, it has been found that sperm function is linked to the quality of the sperm genome machinery, which exhibits considerable DNA fragmentation (Chan *et al.*, 2001).

Nonobstructive azoospermia and severe oligospermia related to testicular failure are two of the most difficult types of male infertility to treat. A number of inherited and acquired disorders may result in spermatogenic failure (Chan & Schlegel, 2000).

Microdissection testicular sperm extraction (micro-TESE) that Schlegel (1999) initially developed to select the most appropriate seminiferous tubules that may contain sperms in NOA patients using a Surgical Microscope (Magnification of 20-25x). During TESE, seminiferous tubules carrying spermatozoa may be seen using an operational microscope (magnification 15-259). Micro-TESE provides a number of benefits, including

larger spermatozoa yield per biopsy, reduced testicular tissue removal, and the identification of blood vessels to reduce vascular damage (Schlegel, 1999). This treatment has been extensively proposed as a better way of sperm retrieval in NOA patients, and various studies have validated its superiority for testicular sperm retrieval. When micro-TESE is used on NOA patients, the sperm retrieval rate is reported to be 43-63 percent (Schlegel, 1999; Okubo *et al.*, 2002). It should be emphasised that the surgeon's experience influences the SRR of micro-TESE, particularly in patients with Sertoli cell-only syndrome (SCO) (Ishikawa *et al.*, 2010). To treat these individuals with severe infertility, experienced embryologists and Andrologists are necessary.

So, the current research intends to analyse the hormonal changes of FSH-Testosterone and E2 for the patient and evaluates the output result of fresh and histopathological after surgery to assess the validity of both analyses

MATERIALS AND METHODS

Study Population:

This study was conducted on 53 nonobstructive azoospermia patients. Diagnosis of Azoospermia was established in all patients by analysing at least two separate centrifuged ejaculate specimens in accordance with WHO recommendations.

Micro-TESE:

Collection:

TESE was carried out in accordance with the method described by Silber et al (1996). After the testicle was stabilised, a tiny incision was made in the testicle's midsection, cutting through the scrotal skin, tunica vaginalis, and tunica albuginea. A significant portion of the extruding testicular tissue was removed using little scissors, rinsed with medium to eliminate blood traces, and put in a Petri dish containing 1-3 ml of sperm washing media. Testicular Sperm Extraction (TESE) and Fine Needle Aspiration were among the biopsies performed (FNA).

The Surgeon decided the type of Maneuver.

For TESE, the embryologist receives the selected cut tissue ina Petri dish, it is completely dissected by Forceps and well-washed by Sperm Wash media, and the fluid is primarily scanned. (Fig. 9 a, b).

For FNA, the embryologist receives the aspirated tubules in sperm Wash media, and the fluid is primarily scanned.

Processing:

1- A wet preparation was performed in the laboratory for evaluation (Jow *et al.*,1993). the testicular tissue was placed in a petri dish containing 1.5ml of culture media MHM-C (Multiple purpose handling media-complete, Irvine Scientific). Using two Forceps, two glass slides, or two insulin syringes, testicular tissue was vigorously shattered and chopped (S-S disposable syringe, SUNG shim medical make in Korea) until a thin slurry of dissociated tissue was formed, then inspected under an inverted microscope. The surgical process was discontinued after spermatozoa were discovered. If no spermatozoa were found, biopsies were collected from other locations of the same testicle as well as the contralateral testicle. A portion of the testicular material was submitted for histological investigation to determine the existence of spermatozoa.

2- Fluid with tubules of biopsy was centrifuged at 1300 rpm for 5 min.

3-The supernatant was removed, then 1 ml of Erythrocyte- Lysing Buffer (ELB) containing 155 mM NH_4Cl, 10 mM $KHCO_3$, 2 mM EDTA, pH 7.2) on the pellet for 5 minutes.

4- Add 2ml of culture media, then Centrifuge at 1300RPM for 10 min (if the sample is too bloody, repeat the ELB method)

5- Supernatant removed except for 0.15 or 0.2 based on expected concentration.

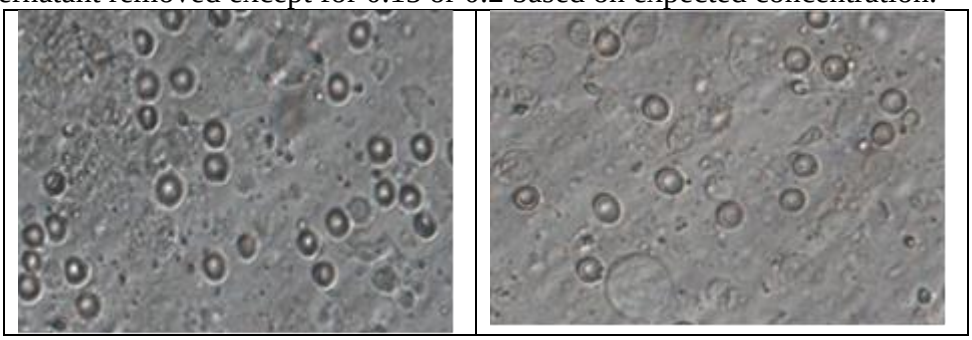


Fig. 1: a. and b. Testicular Biopsy before Processing.

6- After Processing We are using a petri dish (IVF Dish 60×15mm, Spllife Sciences) containing multiple droplets from a sample (up to 50) of 10 microns, all droplets mixed with culture media and Sperm Mobile (GM501 Sperm Mobile-GYNEMED -Germany), Which Activating Sperms.

7- the sample covered by mineral oil (GM 501Mineral Oil - GYNEMED, Germany).

8- sample was examined on an inverted Microscope (Inverted Microscope-NIKON ECLIPSE TI).

9- During searching, the sample is sure to contain only SCO cells or Spermatocytes, including Primary and Secondary Spermatocytes; we can also find early spermatid, late spermatid, and spermatogenesis.

Examination of Testicular Biopsies and Histopathology:

The eligible subjects included in this study were already subjected to the following: Full history taking and physical examination was done.

Investigations:

Serum total testosterone, FSH and E2 levels were assessed within 2 months before the micro-TESE attempt.

Micro-TESE:

The micro-TESE method has already been described by Schlegel (1999). A midline scrotum incision was done, and the testis was delivered, the tunica vaginalis was opened to reveal the tunica albuginea. The tunica albuginea was extensively opened in an equatorial plane, under an operating microscope, with a subtunical vessel preservation.

After opening the tunica albuginea, the testicular parenchyma was examined under an operating microscope at magnifications ranging from 20 to 25. The examination included microdissection deep into the testicular parenchyma to include examination of as much testicular tissue as was required until spermatozoa were discovered, or to offer tissue collection for Histology examination after the procedure if no spermatozoa were identified intraoperatively.

The Surgeon selected Small samples (1–15 mg) of opaque distended tubules within the testicular parenchyma. Then embryologist tested the samples for spermatozoa.

the surgeon used to do Micro-TESE on all patients and the samples extracted from the patient with divided into two halves.

The first half was examined by the Andro lab, and if it is positive, the second half with collected by the Andro lab.

However, if it was negative sperm retrieval the second half was sent to the Histopathology lab.

Histopathology:

Bouin's solution was used to preserve testicular biopsy fragments right away. Semi-thin paraffin wax slices (4 m) were stained and studied under light microscopy at 3400 magnification using conventional procedures.

The slides were reviewed by three assessors who were not informed of the TESE findings or the detection of spherical spermatids. Hypospermatogensis (a decrease in the number of normal spermatogenic cells), maturation arrest (the absence of the latter stages of spermatogenesis), Sertoli cell-only (the lack of germ cells in the seminiferous tubules), and localised spermatogenesis (small areas of apparently normal spermatogenesis) were the classifications of testicular histology.

Statistical Data Analysis:

Various applications were used to examine data from 53 patients. Excel 365 was utilised for data input and data visualisation. Before doing any statistical analysis, the data was cleansed. The data was initially examined using IBM SPSS VER.25. The frequency of the major quantitative variables has been determined, as have descriptive statistics for the important quantitative variables. Inferential statistics were employed to address the study's primary questions. All parametric variable assumptions have been checked. The Mann-Whitney test was used to compare independent two-group variables, whereas the Wilcoxon signed-rank test was employed to compare correlated groups. Data were reported as means and quartiles, with a P-value of 0.05 deemed significant. Data were tested for satisfying assumptions of parametric tests, continuous variables Shapiro- Wilk, and Kolmogorov-Smirnov test results for normality. However, results showed that variables followed a non-normal distribution pattern, so the data transformation was utilized to meet normality requirements for parametric analysis. Spearman correlations were used for testing the correlation relationship between parameters using Minitab V 21.2

RESULTS

Table (1) shows the patient Data distribution with ages ranging from (20 to 52) years old, while the recorded value for FSH fluctuated between (1.7 to 68 IU/L). Testosterone gets low as (<2.5 to 1406 ng/dl). Finally, the calculated value for E2 started from (4.84 to 214 pg/ml).

Table 1: Patient data distribution.

	N	Mean	Std. Deviation	Minimum	Maximum
Age	53	33.49	7.455	20	52
FSH	53	21.95	15.14	1.7	68
Testosterone	53	548.15	277.72	<2.5	1406
E2	53	37.61	35.49	4.84	214

Five stages have been recognized for fresh and histopathology examination post-operatively (SCO- Spermatocyte- Early spermatid - Late Spermatid and Hypospermatogensis). (9) cases out of (53) patients have non-typical results between both groups, in these cases, Hypospermatogensis(Positive Sperm Retrieval) were observed in histopathology results. Spermatocyte was the most common for Fresh and histopathology analysis (43 and 31% respectively out of the 53 cases) **(**Fig. 1**)**.

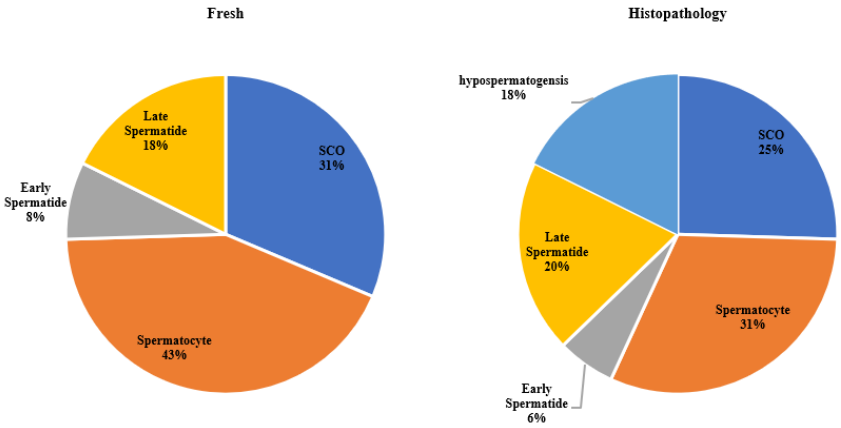


Fig. 1: Pie chart representing Fresh and histopathology results.

The patients had been subclassified according to their age into six age classes with 5 years intervals (20-24; 25-29; 30-34; 35-39,40-44 and 45-50) to evaluate if one of the hormones has predicting ability for sperm retrieval rate as related to the age of the patient. We found that hormonal changes follow the uneven trend with FSH recorded. its lower average for the age class (20-24) years (6.15 IU/L) with the highest mean recorded for the age class (40-44) years (42 IU/L). As regard the serum Testosterone level, the lowest analysed serum total testosterone was observed with age class (45-49) years (317.67 ng/dl) and the highest at age class 30-34 years (602.31 ng/dl). Finally, E2 follows the FSH with that the lowest and highest values observed at the same mentioned age classes (20-24) years and (40-44) years (21.25 and 49.13 pg/ml respectively) (Fig. 2). Figure (3) represents the role effect of hormones throughout age classes, revealing that E2 was the most reliable hormone in which age classes differ following FSH than Testosterones.

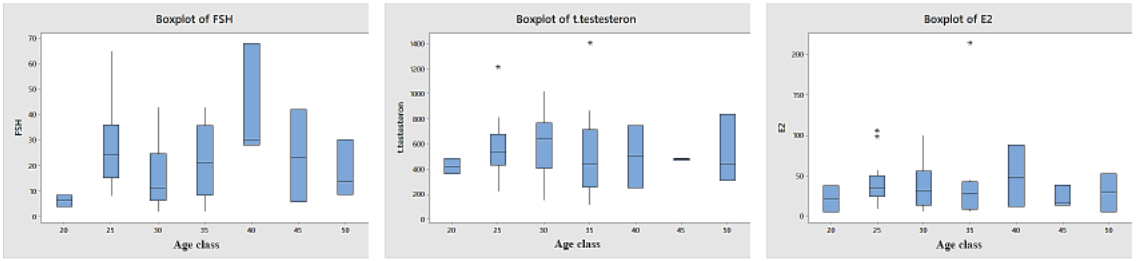


Fig. 2: Boxplot representing quartiles, mean, and range of tested hormones according to age classes.

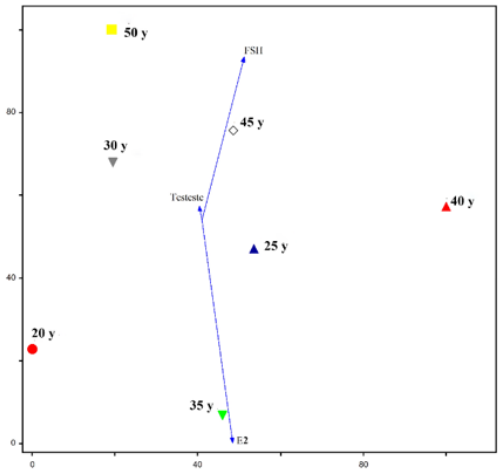


Fig. (3): PCA ordination shows the effect of age on tested hormonal values.

Figure (4) shows Fresh and histopathology results obtained according to the age class revealing the typical and non-typical cases and at any age class they appear. All age classes show one or several nontypical cases appears except for age classes (20-24) and (45-49) years old.

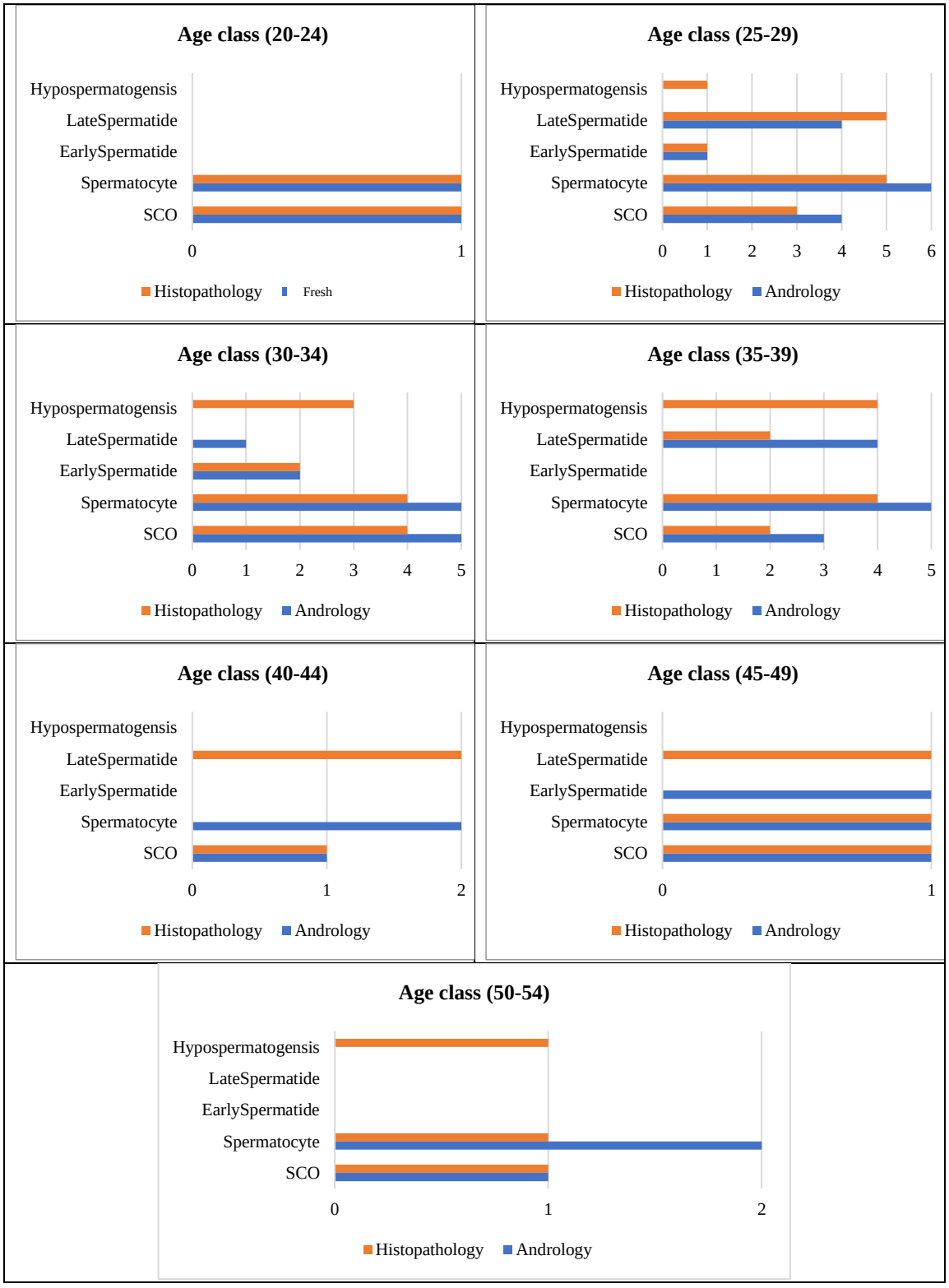


Fig. 4: The bar chart shows the Fresh and histopathology results according to age class**.**

Tables (4 & 5) the Size effect analysis (Eta^2) revealed the size effect of age and hormones on Fresh and histopathology results. A large effect was shown on both Fresh and histopathology results as the Eta value exceeded 0.138.

Table 4: Eta (nominal by interval analysis) for Fresh result.

Eta	Dependent Variable	Value	E^2
Nominal by Interval	Fresh vs. age	0.601722	0.362069
	Fresh vs. FSH	0.934594	0.873465
	Fresh vs. Testosterone	0.948989	0.90058
	Fresh vs. E2	0.876748	0.768687

Table 5: Eta (nominal by interval analysis) for histopathology result.

Eta	Dependent Variable	Value	E^2
Nominal by Interval	Histopathology vs. age	0.636773	0.40548
	Histopathology vs. FSH	0.872712	0.761626
	Histopathology vs. Testosterone	0.905591	0.820095
	Histopathology vs. E2	0.822984	0.677303

Paired sample correlation shows a positive correlation between Fresh and histopathology results with only a 0.673 correlation value. In addition, paired sample t-test revealed a significant variation (P< 0.05) between observed Fresh and histopathology results (Tables 6 & 7).

Table 6: Paired sample correlation between Fresh and histopathology result.

		N	Correlation	Sig.
Pair	Fresh & Histopathology	53	.673	.000

Table 7: Paired sample test for Fresh and histopathology result.

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair	Fresh - Histopathology	-.58491	1.08191	.14861	-.88312	-.28669	-3.936	52	.000

As shown in Figures (5 & 6) the hormonal test mean, Std, and range for patients, according to Fresh results, revealed a semi-bell-shaped trend for Testosterone and E2. At the same time, FSH follows an uneven trend with a peak for spermatocyte patients.

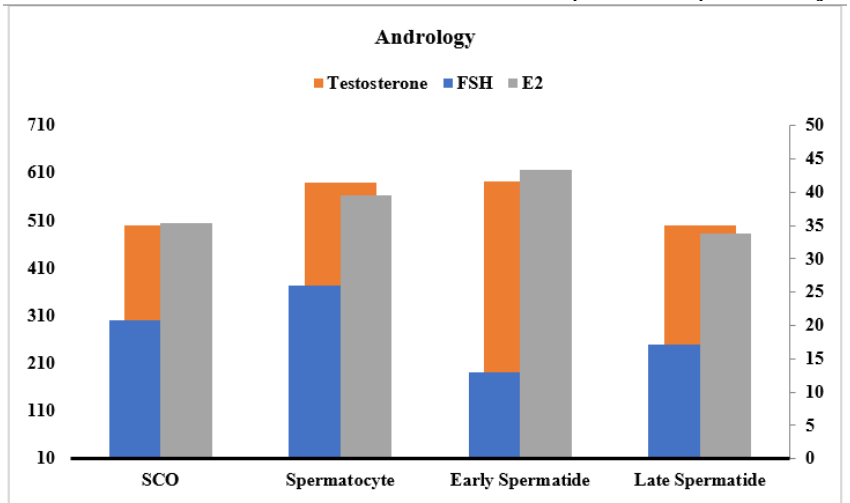


Fig. 5: Line chart shows mean hormonal value according to Fresh result.

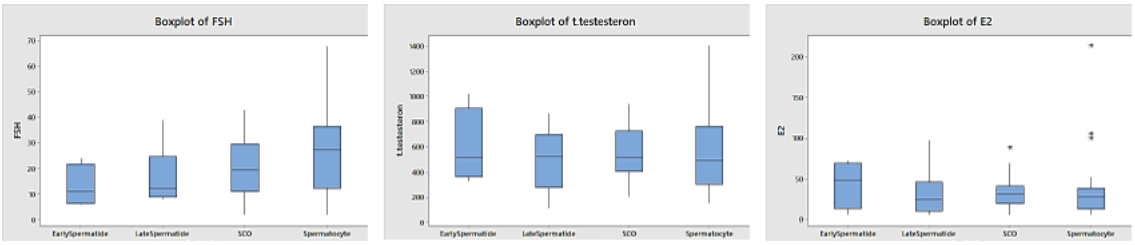


Fig. 6: Boxplot representing quartiles, mean, and range of tested hormones according to Fresh result.

As shown in Figures, (7 & 8) the hormonal test mean, Std, and range for patients according to histopathology results revealed an uneven trend with a peak for FSH for late spermatids patients; at the same time, mentioned patients shows a lower peak for Testosterone and E2.

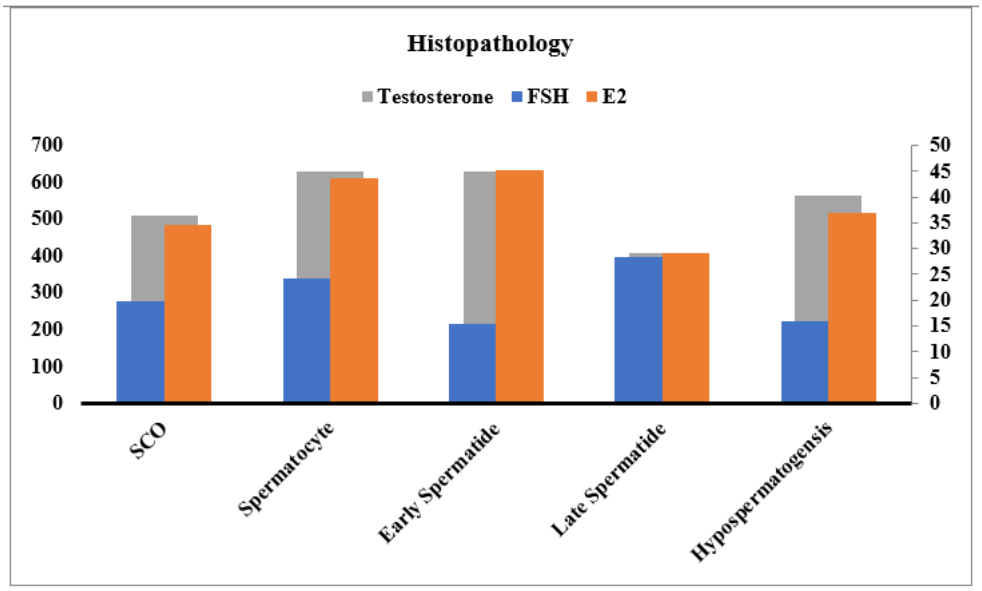


Fig. (7): Line chart shows mean hormonal value according to Histopathology result.

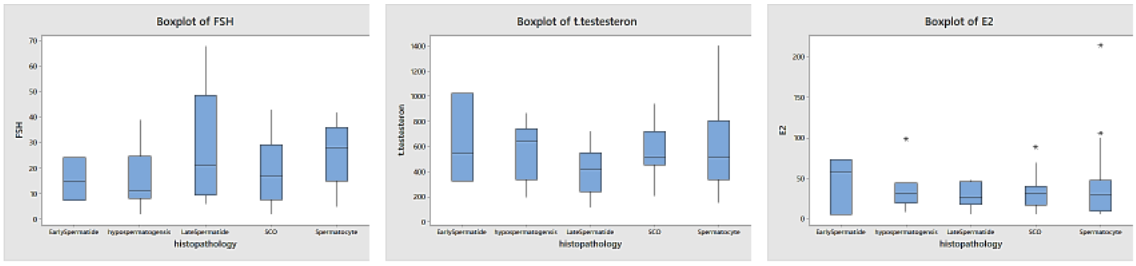


Fig. 8: Boxplot representing quartiles, mean, and range of tested hormones according to histopathology result.

Nine cases show hypo spermatogenesis in histopathology, while they represent different results in fresh examination **(**Fig. 9).

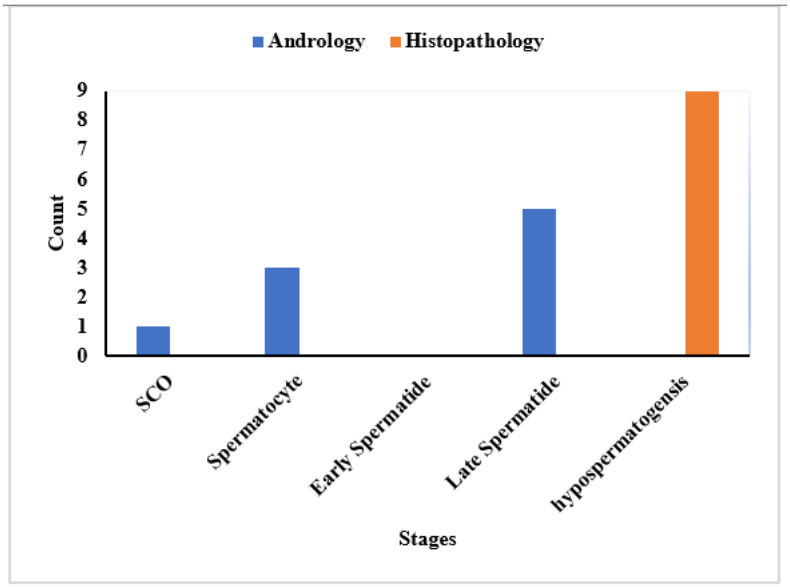


Fig. 9: fresh result in relation to positive histopathological examine nontypical cases.

Figure (10) shows a peak for FSH and E2 for age class 40-44, while a peak formed for Testosterone at age class 50-54 (Fig. 10).

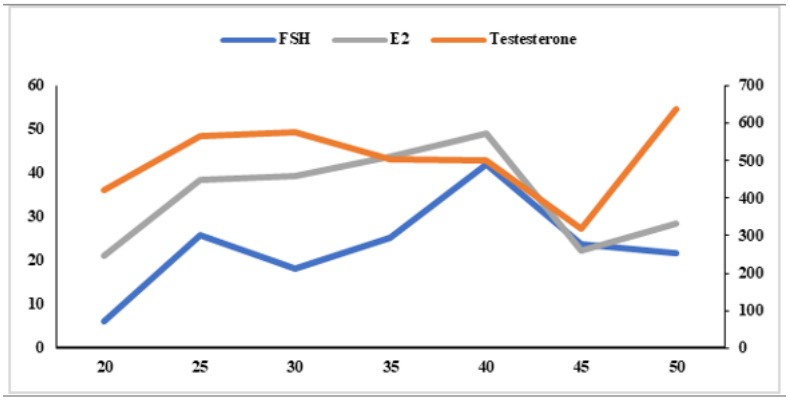


Fig. 10: The line chart shows the mean hormonal value for typical cases according to age classes**.**

Figure (11**)** shows a high peak for Testosterone at age (30-34) and a high peak for FSH and E2 at age (25-29). At the same time, a Low peak was recorded for all hormones at age (50-54) except for E2, which shows a low peak at age (30-34).

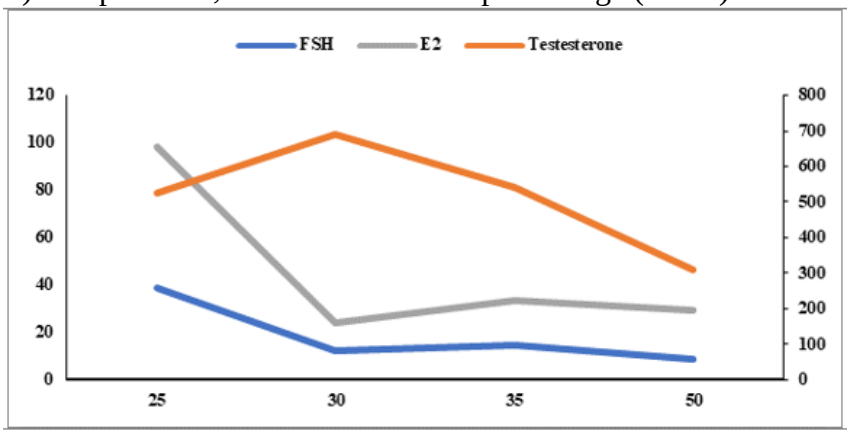


Fig. 11: The line chart shows the mean hormonal value for nontypical cases according to age classes.

Testosterone shows a significant positive Correlation (0.724) with E2 in typical cases, while FSH was correlated positively with E2 in nontypical cases (0.636), but it was no significant correlation (Tables 12 & 13).

Table 12: Correlation relationship between hormonal values for typical cases.

		Age	FSH	Testosterone	E2
Age	Pearson Correlation	1			
	Sig. (2-tailed)				
FSH	Pearson Correlation	0.18	1		
	Sig. (2-tailed)	0.254			
Testosterone	Pearson Correlation	-0.103	-0.261	1	
	Sig. (2-tailed)	0.516	0.087		
E2	Pearson Correlation	-0.014	-0.079	.724**	1
	Sig. (2-tailed)	0.929	0.617	<.001	
***** Correlation is significant at the 0.05 level (2-tailed).***					

Table 13: Correlation relationship between hormonal values for nontypical cases

Correlations		Age	FSH	Testosterone	E2
Age	Pearson Correlation	1			
	Sig. (2-tailed)				
FSH	Pearson Correlation	-0.39	1		
	Sig. (2-tailed)	0.3			
Testosterone	Pearson Correlation	-0.308	-0.052	1	
	Sig. (2-tailed)	0.421	0.894		
E2	Pearson Correlation	-0.302	0.636	0.106	1
	Sig. (2-tailed)	0.43	0.066	0.787	
***** Correlation is significant at the 0.05 level (2-tailed).***					

Andrology Result:

Primary spermatocyte is characterised by circular cells of 13-21M, a granulated nucleus with 1/4 or 1/3 of cell size, and a central or sub-central nucleus Fig(12). Secondary spermatocyte with two stages; the first stage is characterized by circular cells of 9-12M, a granulated nucleus with 1/4 or 1/3 of cell size, and a central or sub-central nucleus (Fig.13). At the same time, the second stage referred to as secondary in division spermatocyte is characterized by elongated cells with 2 nuclei (circular, granulated& peripheral) (Fig.14). The Early spermatid has elongated or round cells with a Condensed nucleus (>5 Mm) (Fig.15). Late spermatids are defined by elongated cells with a condensed nucleus (<5 Mm) (Fig.16). Encounter, hypo spermatogenesis observed in histopathology featured a head with 4-6 Mm diameter (Fig.17). Finally, SCO (Fig. 18).

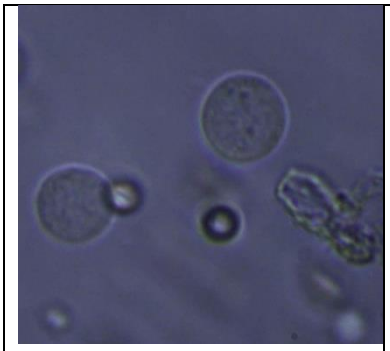


Fig. 12: Primary spermatocyte

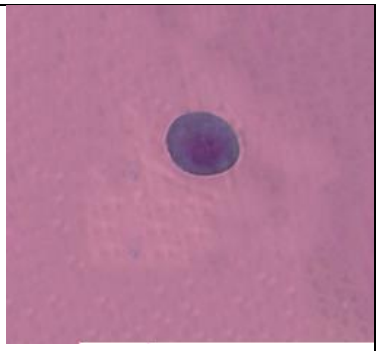


Fig.13: Secondary spermatocyte

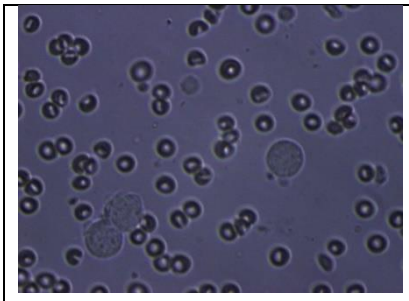
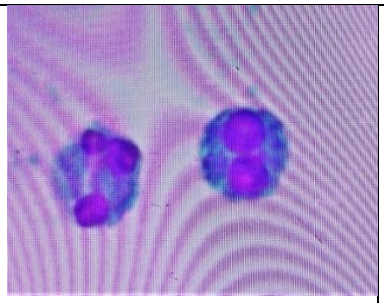


Fig. 14: Secondary in-division spermatocyte



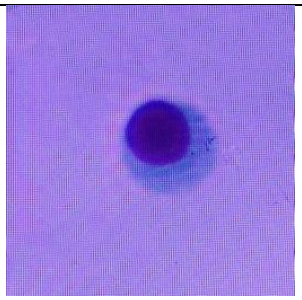


Fig.15: Early spermatid

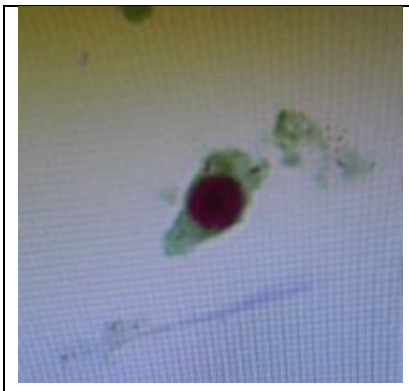


Fig. 16: Late spermatid

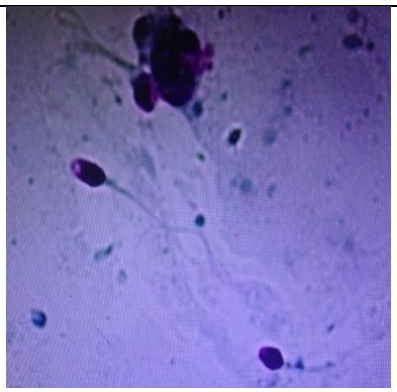


Fig.17: Hypo spermatogenesis or sperm

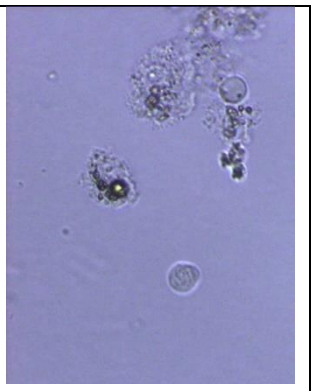


Fig. 18: SCO

DISCUSSION

A testicular biopsy is an important diagnostic and predictive tool in reproductive medicine for assisted reproductive technologies (ARTs) and the risk of testicular neoplasia. Andrology tests cannot distinguish between obstructive azoospermia (OA) and non-obstructive azoospermia (NOA). Currently, variable histological reporting techniques and the use of unclear wording reduce the usefulness of the research on TESE recovery rates, making it difficult to evaluate treatments and explore genotype-phenotype relationships (McLachlan *et al.*, 2007).

The incidence of male infertility and the subsequent histological findings in testicular biopsies vary significantly across the globe due to a variety of underlying etiological factors, including social practices, genetic causes, and environmental conditions such as underlying infections, chemicals, radiation, and heat exposure (Saradha and Mathur 2006 & Lunenfeld and Steirteghem 2004)

There are three basic groups of Azoospermia causes: pre-testicular, testicular, and post-testicular (Wong *et al.*, 1978). Extragonadal etiologies such as hypothalamic, pituitary, or adrenal endocrine disorders, chronic illnesses such as diabetes mellitus and hypertension, and certain medicines are all pre-testicular causes of infertility. Post-testicular causes include duct obstructions draining the testes caused by congenital trauma, surgery, and Anomalies in spermatogenesis are examples of testicular causes (Wong *et al.*, 1978) The distinction between post-testicular obstructive and pre-testicular or testicular nonobstructive causes of male infertility is critical because men with obstructive etiologies may have more cost-effective treatment choices, such as microsurgical reproductive system restoration (Rashed *et al.*, 2008).

Testicular biopsy is still the most important inquiry for all testicular causes of infertility (Levin, 1979). It is not the sole factor that determines the testicular histopathological pattern, but it is the most accurate predictor of whether or not sperm may be found in the testis for therapeutic sperm retrieval in assisted reproductive procedures (McLachlan *et al*., 2007).

In the current investigation, 53 men with Non-Obstructive Azoospermia had bilateral testicular biopsy extracted for sperm retrieval, although only nine (16.98 percent) of these patients revealed a discordant pattern to fresh results, which was similar to what has been reported by *Abdullah and Bondagji* (2011). Paired sample correlation shows a positive correlation between fresh and histopathology result with only a 0.673 correlation value. Furthermore, the paired sample t-test demonstrated a significant difference (P 0.05) between the observed fresh and histopathological data.

Patients underwent three Hormonal analyses including FSH, testosterone and E2. FSH recorded its lower average for age class 20-24 years (6.15 IU/L) with the highest mean recorded for age class 40-44 years (42 IU/L), Testosterone on the other hand, recorded its lowest calculated value with age class 45-50years (317.67 ng/dl) and the highest at age class 30-34 years (602.31 ng/dl). Finally, E2 follows the FSH with that the lowest and highest values observed at the same mentioned age classes 20-24 years and 40-44years (21.25 and 49.13 pg/ml respectively).

In the present study, we used the Size effect analysis (Eta^2) to evaluate the most factor affecting the positive sperm retrial rate, we found that the serum total testosterone level was the most which have predictor for sperm retrieval rate. the size effect of age and hormones on fresh and histopathology results. A large effect was shown on both results obtained from fresh and histopathology as Eta value exceeded 0.138. revealing the role of hormonal levels in the patient's final result.

There is presently no agreement on how to record testicular biopsies in a systematic manner; language is not defined and is often subjective and inaccurate. The presence and amount of spermatogenesis, maturation of germinal cells, and the presence of tubular atrophy, interstitial fibrosis, and Leydig cell hyperplasia are the most common histological classifications of a testicular biopsy (Nistal and Paniagua 1999 & McLachlan *et al*., 2007)

Hypospermatogenesis was represented in 9 cases, (16.98%) in histopathology results in whom we couldn't retrieve sperms in the fresh examination. This finding was lower than what has been reported by Abdullah and Bondagji (2011) who stated 29 cases, (29%) in 2011. Similarly, our findings were lower than those of Jamal and Mansoor (2001), who found a 25% occurrence. Haddad *et al.* (2004) discovered a significant prevalence of hypospermatogenesis (55.8 percent). Meinhard *et al.* (1973) and Colgan *et al.* (1980) both reported a 46 and (49 percent) incidence of hypospermatogenesis, respectively. Thomas (1990) and Wong *et al.* (1978) reported 19 and (23 percent, respectively). Jamali and Haeri (1999) from Iran found a 36.6 percent incidence with a mean age of 32.4 years, which varies from two previous studies (Al-Rayess and Al-Rikabi 2000 & Thomas and Jamal 1995) that indicated a considerably lower incidence of hypospermatogensis. In a study of 230 testicular biopsies, Al-Rayess and Al-Rikabi (2000) from Riyadh found a (13%) incidence of hypospermatogenesis, whereas Thomas and Jamal (1995) from the western part of Saudi Arabia found a (3.7%) incidence of hypospermatogenesis.

The absence of spermatogonia and primordial spermatids characterises hypospermatogenesis. In other words, all stages of spermatogenesis exist, but their quantities are reduced (Nistal and Paniagua 1999). Hormonal dysregulation, congenital germ cell insufficiency, androgen insensitivity, chemical exposure, and heat and radiation exposure are all clinically connected to hypospermatogenesis (Greenhall and Vessey

1991). Disparities across studies may be explained by differing criteria for selecting patients for biopsies.

The incidence of early and late spermatids in the present study was 3 cases, (5.7%) and 10 cases, (18.9%) for histopathology results respectively. While 4 cases, (7.5%) were labeled as early spermatids and 9 cases, (17%) were labeled as late spermatids within the fresh result. The incidence was comparable to that reported in various studies (Al-Rayess and Al-Rikabi 2000; Thomas and Jamal 1995), although much lower than worldwide numbers. Nagpal *et al.* (1993) and Jamali and Haeri (1999) The incidence of GCMA cases was (28%) in research by Rashed et al., (2008) from Egypt, whereas Thomas (1990) from Jamal and Mansoor (2001) Saudi Arabia reported low occurrences of 5 and (7%), respectively. Haddad *et al.* (2004) identified a relatively low incidence of GCMA (1.7 percent) (2004).

In our study, 13 instances (16%) of SCO were found in histopathology results, whereas 16 cases (30.2%) were found in fresh results. This result is consistent with three previous investigations. (Nagpal *et al*. 1993; Jamal and Mansoor 2001 & Jamali and Haeri 1999) in which comparable data were recorded. Jamal and Mansoor (2001) from Saudi Arabia's western area reported an incidence of (16.5 percent), whereas Thomas and Jamal (1995) from the same region reported an incidence of (27.2 percent). Al-Rayess and Al-Rikabi (2000) from Riyadh, Saudi Arabia, observed a comparable high prevalence (39 percent).

There were nine conflicting occurrences (9 cases) between fresh and histology in the present investigation. These patients were classified as hypospermatogenesis by histopathology, although fresh revealed one instance of SCO, three cases of spermatocyte, and five cases of late spermatid.

To the best of our knowledge, no previous research has been conducted to analyse the biopsy from NOA patients who have negative sperm retrieval in a fresh examination on the side of histological evaluation. The goal of this research is to examine and assess the differences between fresh and histopathological examinations in order to put our Andro lab findings to the test in such individuals. In our study, we discovered that 16.9% of the patients had sperms in histology but could not retrieve sperms in Andro lab. These findings may point us in the direction of repeating a testicular biopsy investigation.

Differences may also be attributable to the methods of histological preparation of the testicular biopsy, which provides additional data about the sample, for example. Bouin's solution was used to preserve testicular biopsy fragments right away. Semi-thin paraffin wax slices were stained and studied under light microscopy at 3400 magnifications using conventional procedures.

Conclusion and Recommendation:

As this study is unique and to our knowledge is the first time to compare both fresh and histopathology so this may be very helpful in the Evaluation and Auditing of Andro labs for their results.

We encourage investigators to do more studies about the difference between fresh and histopathology examination of testicular biopsy with a larger sample size to define the percentage cut off above which means something went to be controlled within the quality operation of Andro lab.

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