Journal of Research Administration
Society of Research Administrators International

MALE INFERTILITY WITH RESPECT TO ITS RISK FACTORS /ULTRASOUNDS AND HORMONAL PROFILE AND HISTOPATHOLOGICAL FINDINGSWITH PREDICTIVE VALUE OF SPERM RETRIEVAL

Authors Details;

1. **Hamza Mohamed**
Department of Anatomy, Faculty of Medicine , Northern Border University , Arar, Saudi Arabia
(hamza.alamin@nbu.edu.sa) https://orcid.org/0000-0002-5666-3140

2. **Abdelrahim Awadelkarim Abdelrahman Mohamed**

Assistant professor ,Department of Obstetrics and Gynaecology , College of Medicine ,University Of Ha'il (aawdelkarim@yahoo.com)

3. **Mohammed M. Mosaed**
[1]Department of Anatomy, Faculty of Medicine , Northern Border University , Arar , Saudi Arabia
[2]Anatomy Department Al-Azhar ,Faculty of Medicine , Al Azhar University Egypt
(mohamed.abdallah@nbu.edu.sa)

4. **Abdelrhman Alyan**
Department of Anatomy, Faculty of Medicine, Northern Border University, Arar , Saudi Arabia
(abdelrhaman31@yahoo.com)

5. **Enas Haridy Ahmed**
Faculty of Medicine , Anatomy Department , Hail University ,Ain Shams University Craio,Egypt(e.hardy@uoh.edu.sa)

6. **Manal Elzein Musa Ismail**
Assistant Professor Obstetrics and Gynecological Nursing, Nursing and Applied Medical Science Buraydah College Burraydah, Saudi Arabia (manal.musa@bpc.edu.sa)

7. **Nagla Hussien Mohamed Khalid**
Faculty of Applied Medical Sciences, Radiological Science Department,Najran University, SaudiaArabia(najlabashab@yahoo.com)

8. **Omnia Abdalla Mahmoud Higazy**
Assistant Professor, Medical surgical nursing, College OF Nursing, Jazan University Saudi Arabia (omniahigazy@gmail.com)

[9.] **Amal Daher Alshammari**

Department of family Medicine, College of Medicine, University of Ha'il, SaudiArabia(dr.amal.1405@gmail.com)

Coressponding Author;

Fahmida Khatoon *

Associate Professor, Department of Biochemistry, College OF Medicine, University OF Ha'il, Saudi Arabia

Email;drfahmida24@gmail.com

Orcid id; 0000-0002-1120-408X

Abstract

Background: To provide indicators for the likelihood of sperm retrieval in patients undergoing testicular sperm extraction is a major issue in the management of male infertility by TESE. The aim of our study was to determine the impact of different parameters, including testicular histopathology, on sperm retrieval in case of reoperation in patients undergoing testicular sperm extraction.

Methods: We retrospectively analyzed patients who underwent sperm extraction for intracytoplasmic sperm injection and testicular biopsy. Histology was classified into: normal spermatogenesis; hypospermatogenesis (reduction in the number of normal spermatogenetic cells); maturation arrest (absence of the later stages of spermatogenesis); and Sertoli cell only (absence of germ cells). Semen analysis and serum FSH, LH and testosterone were measured.Patients were selected from Sindh institute of reproductive medicine with the help of an Uroandrologist. The initial step was the assessment of sub fertile men presenting in infertility clinic

Results:. There were 63% successful sperm retrieval. Higher testicular volume, lower levels of FSH, and better histological features were predictive for sperm retrieval. The same parameters and younger age were predictive factors for shorter time for sperm recovery. After multivariable analysis, younger age, better semen parameters, better histological features and lower values of FSH remained predictive for shorter time for sperm retrieval while better semen and histology remained predictive factors for successful sperm retrieval. The predictive capacity of a score obtained by summing the points assigned for selected predictors (1 point for Sertoli cell only, 0.33 points for azoospermia, 0.004 points for each FSH mIU/ml) gave an area under the ROC curve of 0.843.

Conclusions: This model can help the practitioner with counseling infertile men by reliably predicting the chance of obtaining spermatozoa with testicular sperm extraction when a repeat attempt is planned.

Keywords: FSH; Semen; Sperm retrieval; Testicular biopsy; Testicular sperm extraction (TESE).

INTRODUCTION

Infertility is complex and it has multiple causes and consequences on the affected people depending on the gender, sexual history, lifestyle, society, and cultural background.[1] Infertility is a global public health concern partly due to its complexity and to difficulty in prevention, diagnosis and treatment. A couple is considered clinically infertile only when pregnancy has not occurred after at least twelve months of regular sexual activity without the use of contraceptives.[2, 3, 4]

Infertility affects about 8 percent to 15 percent of the world's population and in about half of the cases men are either the single cause of, or contribute to, the couple's infertility.[2, 3, 5, 6] In spite of the significant contribution to infertility, males are never pushed up for fertility evaluation in Pakistan where potency is considered a proof of normal fertility. The prevalence of infertility in Pakistan is 21.9%.[7] Problem of infertility is the same in Pakistan as in the rest of the worldbut the desire of having a child is much more intense in this part of the world.[8]

We are now in the era of recognition of the importance of the male factor in the consideration of why a couple has been unable to have desired children. Qualitative or quantitative abnormalities in sperm production occur in 40% to 50% men in reproductive age group.[6,7,8,9,10] Sperm may be immature, abnormally shaped, or unable to move properly. Normal sperm may be produced in abnormally low numbers (oligospermia) or seemingly not at all (azoospermia). This problem may be caused by many different conditions includinginfections or inflammatory conditions, endocrine or hormonal disorders and pituitary problem. Immunological disorders in which some men produce antibodies to their own sperm, environmental and lifestyle factors as well as genetic diseases can also be the root of it.[10]

Azoospermia (absence of sperms in ejaculated semen) is the most severe form of male factor infertility and is present in approximately 5% of all investigated couples.[5] The initial diagnosis of azoospermia is made when no spermatozoa can be detected on high powered microscopic examination of centrifuged seminal fluid on at least two occasions. TheWorld Health Organization (WHO) Laboratory Manual for the Examination of Human Semenand Semen-Cervical Mucus Interactionsrecommends that the seminal fluid be centrifuged for 15 minutes at a standardized centrifugation speed.[6, 8, 10] Azoospermia is currently classified as obstructive and non-obstructive.[3] Obstructive azoospermia is the result of obstruction of either the upper or lower male reproductive tract (epidermis, vas defrens, seminal vesicle, or ejaculatory ducts). Sperm production may be normal but the obstruction prevents the sperm from being ejaculated. Normal

sperm production may be verified through testicular biopsy. Causes of obstructive azoospermia include vasectomy, congenital absence of vas deferens (CABVD), scarring from past infections, and inguinal hernia or hydrocele operations. Non-obstructive azoospermia is the result of testicular failure where sperm production is either severely impaired or non-existent, although in many cases sperm may be found and surgically extracted directly from the testicles. Causes of non-obstructive azoospermia include genetic and hormonal disorders, testicular maldescent and torsion, systemic disease (including cancer), drugs, radiation and toxins.[3, 5,9,10]

Literature Review

Reproduction is a normal human event that is absolutely necessary for the survival of the human race. Since the birth of mankind, men and women have been practicing the reproduction process and there have been some who are unable to do that. More than 4.5 million couples experience infertility each year[5]. There have been millions upon millions of couples that have had to cope with infertility throughout the ages.Infertility is therefore not meant to be the normal course of events and will have a cause even though we may not always find one. Infertility affects at least 20-25% of couples who are of reproductive age. This means that at least one in five of the couples you know will be affected by some degree of infertility.[28] Statistics vary but it would seem that around 35% of male are subfertile and at least 2% of men are totally infertile.[29] Furthermore there is a great scientific debate going on just now about evidence suggesting that male fertility is decreasing markedly as a result of modern lifestyles.[30] Many of the infertile couples are famous, historical figures. One of its example is of Henry VIII, a randy sort of ruler, went through wives like fish go through water. Though there are political reasons for some of his six marriages and their dissolutions; in many cases Henry decided to dump his wife because she had not produced a male heir. Medical science would later show that it is the male chromosome that determines gender in offspring.[31] Infertility arises when either one or both members of a couple are sterile or have severely reduced fertility. Sterility of one partner will always render the couple infertile. In many cultures, infertility is considered a shameful condition, something that is not freely discussed. So, not surprisingly, many men and women either do not know or still have misconceptions about the true causes of infertility.[7, 9] Infertility is a major life stressor that affects approximately 10% of United States married couples.[32] Infertile women and men have reported experiencing depression, helplessness, and marital strain.[33]While many couples do not know the true causes of infertility, the consequences are often apparent, especially for women in the developing world. Grief and frustration, guilt, stigmatization and ridicule, abuse, marital instability, economic deprivation, and social ostracism are just some of the consequences that have been reported in various parts of Asia and Africa.[34]Many of these consequences are personal, but others are societal. Throughout the world women are expected to bear children, but these social pressures can be particularly intense in parts of the developing world where voluntary childlessness is rare and opportunities for women, aside from motherhood, are few. In hopes of becoming pregnant, some women who consider themselves infertile may even engage in extramarital relations, a behavior that places them at risk of STDs, including HIV.[35]

When a couple cannot have children, the woman is usually blamed. However, men can be infertile too which is a situation that causes them embarrassment and disappointment. Various myths exist about infertility.

TESTICULAR CAUSES OF MALE INFERTILITY

Chromosomal abnormalities such as Klinefelter's syndrome, XX disorder (sex reversal syndrome), XYY syndrome, Noonan's syndrome (male Turner's syndrome), myotonic dystrophy, bilateral anorchia (vanishing testes syndrome), sertoli-cell-only syndrome (germinal cell aplasia), gonadotoxins (drugs, radiation), orchitis, trauma, systemic disease (renal failure, hepatic disease, sickle cell disease), defective androgen synthesis or action, cryptorchidism, varicocele are included under this heading.[55,56] Several somatic chromosomal abnormalities are associated with male infertility. In a study of 1,263 barren couples, it was found that the overall incidence of male chromosome abnormalities was 6.2%.[57] In a subgroup in which the male partner's sperm count was less than 10 million, the incidence rose to 11%.[59]infertility was often attributed to God's will or psychological problems.[37]

METHODS AND MATERIALS

RESEARCH DESIGN

A cross sectional comparative study was carried out at the department of Anatomy, Institute of Basic Medical Sciences(IBMS)and Dow Diagnostic Research and Reference Lab, Dow University of Health Sciences (DDRRL/DUHS).

SETTING/SOURCES

The samples were collected from the patients attending Sind Institute of Reproductive Medicine (SIRM). The consent for conduction of research and collection of samples was taken from both hospital and patients.

POPULATION OF INTEREST

Subjects for this study presented to infertility clinic with the inability to contribute to a pregnancy despite regular, unprotected intercourse for at least a year with a female partner who had been evaluated by a gynecologist and found to have no structural or functional reproductive abnormality. A total of 1443 consecutive infertile men were investigated between December 2009 till Febuary 2011, out of which 145 were found to be azoospermic. Out of 145 patients, 37 patients had sperm retrieved by percutaneous epididymal sperm aspiration (PESA) so did not required any testicular biopsy. Five patients refused for any of the ART procedure. Finally 103 patients were included in the study. None of the subjects were related to each other and were not on any medication that could affect their reproductive function. No patient had a history of scrotal or inguinal surgery that could injure the vas deferens. All patients underwent a detailed physical examination including a genital examination to rule out congenital absence of vas

deferens. Since none of the patients included in this study had absence of vas, a cystic fibrosis transmembrane regulator gene mutation analysis was not performed.[15]

SAMPLE DESCRIPTION

Study Design;

Cross sectional comparative study.

Sampling Technique;

Non probability, purposive sampling.

Study Duration;

The pilot project was done before the actual research was started. The main research is done from December 2009 till February 2011.

Sample Size Estimation;

With the help of statistician and epidemiologist and in accordance of the hospital turn over record, it was recommended that the sample size should be 100.

STRATEGY FOR SPERM RETRIEVAL

After workup, these azoospermic males were subjected to diagnostic sperm retrieval procedure. Patients were explained regarding various sperm retrieval techniques. After complete understanding of the procedure, informed consent has been taken and then patient was first subjected to percutaneous epididymal aspiration of sperm (PESA) technique. This procedure was carried out as an outpatient procedure in a peaceful and calm environment with good light and comfortable setting. Under possible aseptic technique patient was exposed, clean and draped, anatomical landmarks were identified. First spermatic cord was identified at superficial inguinal ring.[18] About 10 ml of plain 1% lignocaine hydrochloride solution injected along the sides of each spermatic cord near the external inguinal ring. PESA was performed by stabilizing the epididymis between the index finger, thumb and forefinger while cupping the testis with the palm of the left hand, and pushing the tip of a 21 guage butterfly needle through the stretched scrotal skin and into the substance of the epididymis. Negative pressure was created in the system by fully drawing back the plunger of an attached 20 ml syringe containing gamete culture medium (IVF Medium).[19] The tip of the needle was gently moved inwards and outwards within the epididymis until columns of slightly opalescent fluid was seen rising in the needle tubing. When a satisfactory aspirate volume was noted or no more aspirate was obtained, the needle was removed from the epididymis. About 5 ml of this fluid was removed and smeared on a clean dry glass slide before being examined under the microscope to ascertain the presence of live motile spermatozoa. The PESA was repeated maximally three times. It is not uncommon to recover sufficient spermatozoa for ICSI and cryopreservation with the first puncture and aspiration, the

procedure lasted for less than ten minutes. The samples were immediately transferred to the laboratory

RESULTS

Table.1 Frequency distribution of type of infertility in azoospermic males

Type of infertility	**n**	**%**
Primary infertility	95	92.2
Secondary infertility	8	7.8

A total of 103 azoopermic males were enrolled in this study.92.2% of the males were suffering from primaryinfertility,while 7.8% were suffering from secondary infertility.

Out of 103 azoospermic males 89.3% of them were married for once, while in 10.7% of patients this was their second marriage.

ROLE OF BMI

All the subject were analyzed on the basis of BMI categorization and as shown in figure 1.3 almost 83% infertile are with in normal BMI range (18.4-24.9) of control group , however a substantial proportion of infertile males approximately 69% were over weight and 32% obese. (14% BMI > 30)

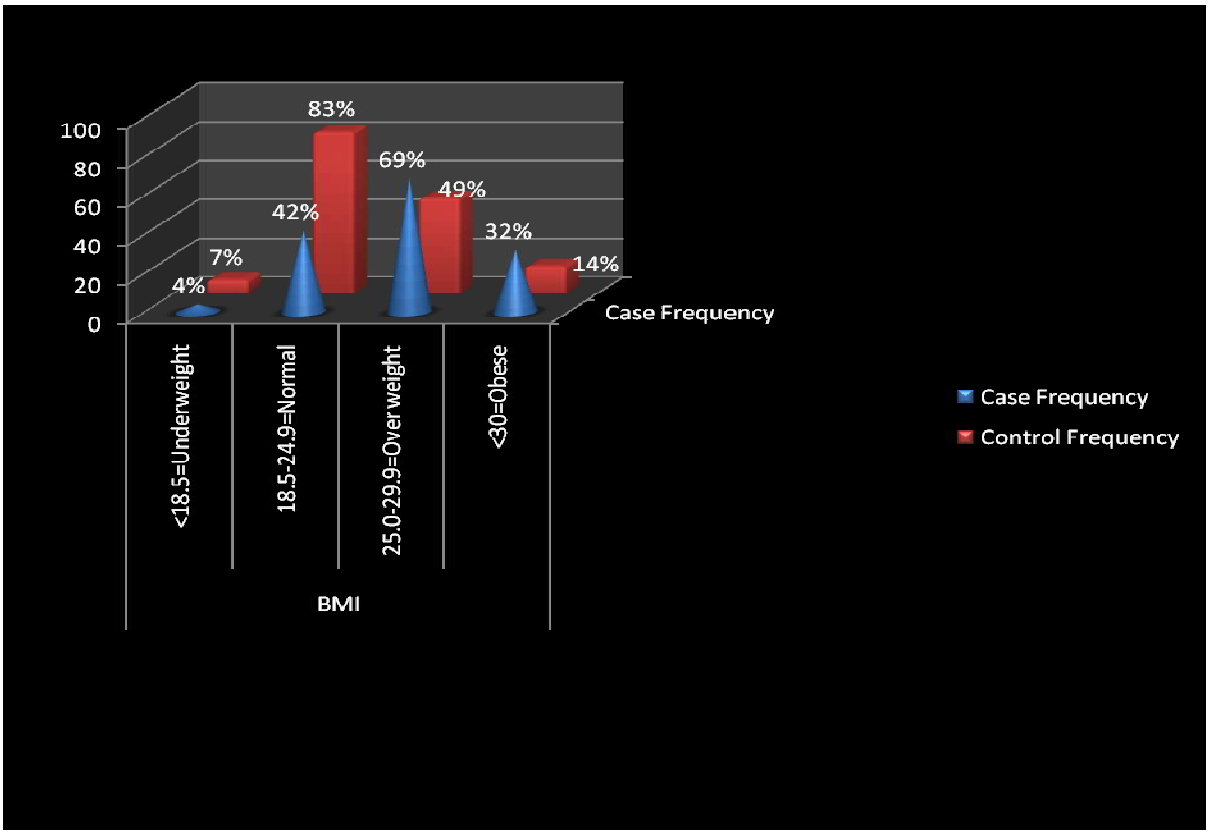


Figure.1.1 Shows distribution of study group according to BMI

Table.3 Frequency distribution of previous children history in azoospermic male

Out of the total of 103 patients 91.3% had never been fathers before while 8.7% of the studied group had a child before and were not able to reproduce after that.

Had any child/children before	n	%
No	94	91.3
Yes	9	8.7

Table.4 Frequency distribution of education status of our study participants

Education status	n	%
Matriculate	6	5.8
Intermediate	15	14.6
Graduate	78	75.7
Post graduate	4	3.9

Regarding education status of the studied population, 3.9% was postgraduate, 75.7% were graduate, 14.6% had done intermediate and 5.8% did matriculation only.

The entire studied group underwent testicular biopsy and the specimen was divided into two parts, one part was shredded with microscopic glass slide and the supernatant was checked for the presence of spermatozoa. The sperm extraction was regarded as positive if an at least single vital spermatozoon was seen. The overall sperm retrieval rate through TESE was 59.2% while in 40.8% of cases sperms were not found.

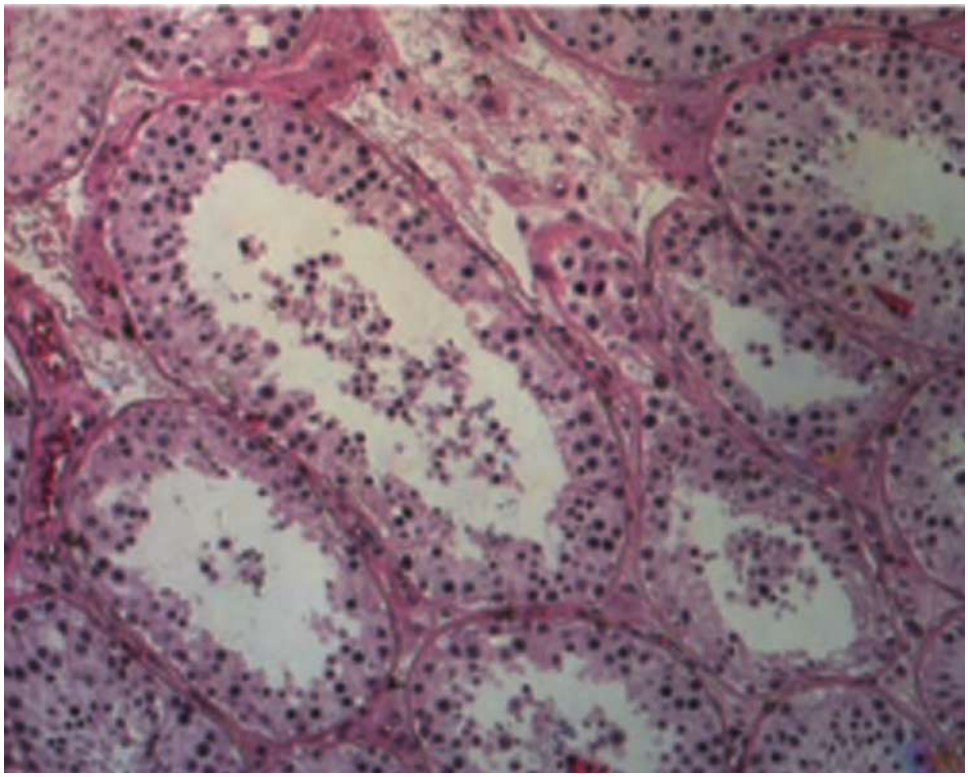


Figure.I Normal spermatogenesis at 10 magnification/hpf.

The seminiferous tubules show a clear lumen and all cell types of germ cell elements were represented. The interstitium was seen containing Leydig cell, blood vessels and sparse fibrous elements. Haematoxylin eosin (HE) stained.

Figure.II Normal spermatogenesis at 40 magnification/hpf.

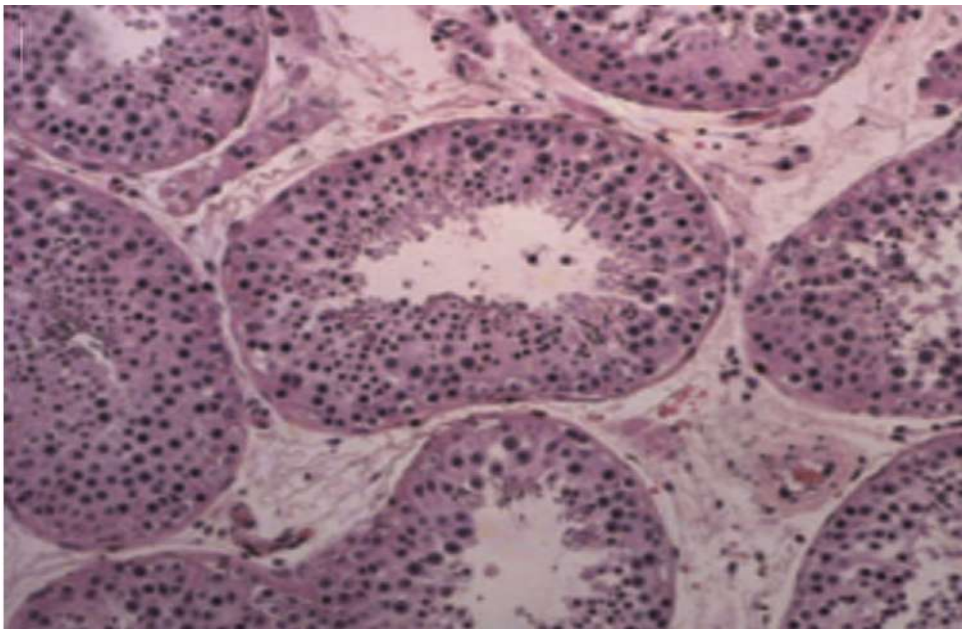


Figure. III Hypospermatogenesis at10 magnification/hpf, features reduced tubule and luminal diameters and a general reduction in germ cell elements but all can be identified. Haematoxylin eosin (HE) stained

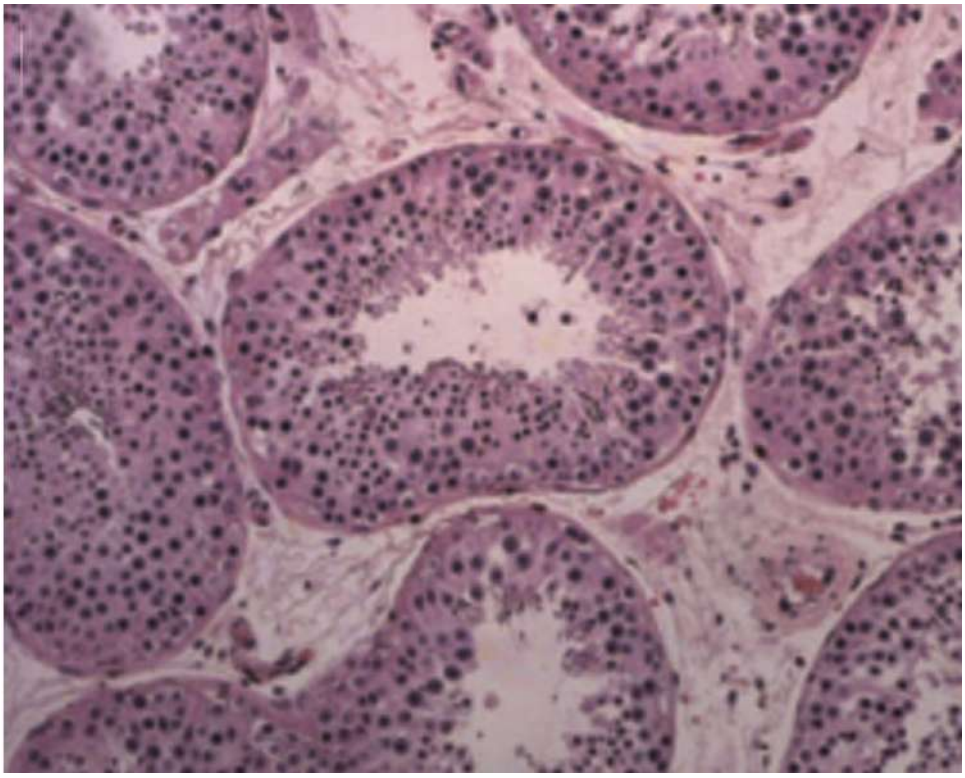


Figure 4Hypospermatogenesis. At 40 magnification/ hpf. Haematoxylin and eosin (HE) stained. High power examination shows a mature spermatozoa pointed by an arrow.

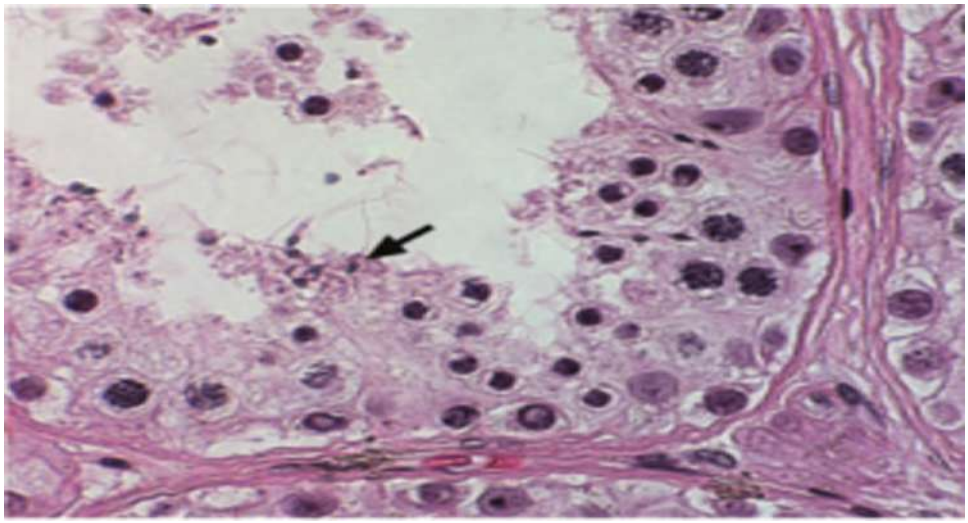
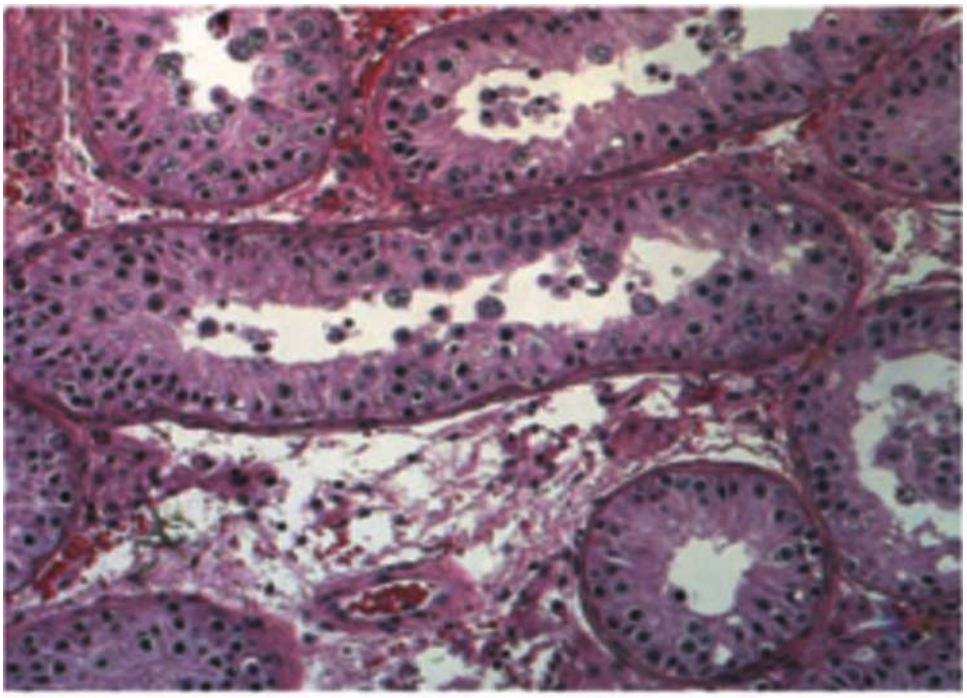


Figure.V Maturation Arrest at 10 magnification/hpf.Section showing reduced tubule diameter and a reduced germ cell complement. Note the absence of mature spermatid,while primary spermatocyte continue to be prominent. Degenerating spermatocytes and cellular debris are being evident. Haematoxylin and eosin (HE) stained



LH and testosterone levels in both groups were insignificantly different and p values were .26 and .58 respectively which were statistically non significant.

Testicular volume was estimated by ultrasound and mean testicular volume was found to be 12.56ml in the success group while 11.61ml in the failure group with a p value of.12 which was statistically non signifant.

Comparison of different histological patterns with FSH levels was done by applying chi square test. It was estimated that in the group 1 , 36% of cases had normal spermatogenesis, 30% of population exhibited hypospermatogenesis,12% of cases with maturation arrest were seen, SCO was evident in 12% of testicular biopsies and 10% illustrates generalized fibrosis. Group 2 had FSH levels of 14mIU/ml and above shows that this group includes 13.2% cases of normal spermatogenesis,30.2% cases had shown hypo spermatogenesis. In 20.8% of cases, maturation arrest and Sertoli cell only pattern were observed while 15.1% of testicular biopsies had shown generalized fibrosis. p value was calculated to be .077 which is non-significant.

BIOPHYSICAL AND ENDOCRINE PROFILES OF STUDIED AZOOSPERMIC GROUP WITH THEIR MEAN(SD) AND 95% CONFIDENCE INTERVAL

Parameters	Mean(SD)	95% CI
Age(years)	33.30±7.2	(32.1,34.48)
Body mass index (BMI kg/m^2)	26.7±4.3	(26.0,27.5)
Follicular stimulating hormone (mIU/ml)	20.7±1.0	(19.0,20.7)
Testesterone (ng/ml)	3.6±3.6	(3.0,4.2)
Prolactin hormone (mIU/ml)	16.9±2.1	(13.5,20.4)

The mean age of the studied group was 26.7±4.3 years with 32.1years as the lower limit and 34.48 years as the upper limit of 95% confidence interval. Body mass index was also calculated after height and weight measurement, which was observed as 26.7±4.3 kg/m2 with 26.0 as the lower limit and 27.5 as the upper limit of 95% of confidence interval. 20.7± 1.0mIU/m was discovered as the mean value for follicular stimulating hormone and had 19.0mIU/ml as the lower limit and 20.7 mIU/ml as the upper limit of 95% confidence interval. Mean Prolactin hormone value was illustrated as 16.9±2.1mIU/ml with 13.5as the lower limit and 20.4 as the upper limit of 95% confidence interval.

DISCUSSION

Extensive changes in the evaluation and treatment of the infertile male have occurred as a result of the technological advance provided by intracytoplasmic sperm injection (ICSI) in 1992.There is evidence from various studies that azoospermic population especially the non obstructiveazoospermic men can be benefitted in fathering their biological children, which would otherwise seems to be impossible[38.]

Testicular biopsy which has been traditionally used as a diagnostic tool in the management of infertility is now used therapeutically to retrieve sperm for intracytoplasmic sperm injection (ICSI). Although less invasive techniques such as testicular fine needle aspiration and percutaneous needle biopsy are efficacious, particularly in cases of obstructive azoospermia, studies in the current literature support that open testicular biopsy is a more reliable method to obtain testicular specimen.[38]

The justification for treating azoospermia caused by germinal failure with TESE and ICSI is based on the consistent surveillance that in a little over 50% of such patients, spermatozoa can be extracted from testicular tissue and that this tiny number of often non-motile testicular spermatozoa can be used[39,40.]

There is, however, limited data available that allows us to adequately counsel such couples as to the success rates of sperm retrieval from SSR. OA and NOA are often used to determine probability of retrieval, but these can only be confidently diagnosed with testicular histology.[137]

Testicular biopsy is now rarely performed prior to SSR, so the use of this terminology in the clinical situation can result in inaccuracies when counseling patients[41].

Conclusion;

Our findingssupport the former results that is the probability of retrieving spermatozoa in testes increased significantly with testicular volume >5 mL.
In the clinical setting, it is important to determineif the etiology of azoospermia is obstructive for tworeasons. Firstly, to identify those that would besuitable for reconstruction, for example vaso-epididymostomy, and secondly to give patients a predictionof their likelihood of sperm recovery. Ashistology is rarely obtained prior to treatment incurrent practice, there is a need for clinical parametersthat can be used to predict the success ofSSR.[41-46]his is especially in light of the emotional andfinancial implications for both partners that areassociated with ICSI. There are factors other thanSSR that must enter into counseling, of course, suchas details of the ICSI procedure, maternal age andtransfer of genetic conditions.

REFRENCES

1. Chuang WW, LoKC, Lipshultz LI, Lamb DJ. Male Infertility. In: Strauss III JF and Barbieri RL. (Eds) Yen and Jaffes Reproductive Endocrinology Elsievier, Saunders, Philadelphia, PA 2004; 669–90.
2. Peter N. Kollettis, M.D. Evaluation of sub fertile man – Am FamPhyisician 2003:67; 2165-73.
3. GR Dohle, GM Colpi, TB Hargreave, GK Papp, AJungwirth, W Weidner.European Urology 2005; 45: 703–711
4. Rowe PJ, Comhaire FH, Hargreave TB, Mahmoud AMA.WHO manual for the standardized investigation and diagnosis of the infertile couple. Cambridge University Press, 2000.
5. Zahra A, Hassan S-u-N, Hassan MS, Parveen N, Park J-H, Iqbal N, Khatoon F and Atteya MR (2022) Effect of physical activity and sedentary sitting time on psychological quality of life of people with and without disabilities; A survey from Saudi Arabia. Front. Public Health 10:998890. doi: 10.3389/fpubh.2022.998890
6. Jones EE, De Cherney AH. The Male Reproductive System. Boron WF and BoulPaep EL (Eds) Medical Physiology, Elsevier, Saunders, Philadelphia, PA.2005; 1122–40.
6. Bhasin S, De Kretser DM, Baker HW. Clinical review 64: Pathophysiology and natural history of male infertility. J ClinEndocrinolMetab 1994; 79:1525–9
7. UNFPA. Pakistan population assessment. Government of Pakistan. 2003.
8. Greenhall E, Vessey M**.** The prevalence of subfertility: a review of the current confusion and a report of two new studies. FertilSteril 1990; 54: 978-983.

9. Kumar R, Gautam G, Gupta NP. Drug therapy for idiopathic male infertility; Rationale versus Evidence.J urol. Oct 2006:176; 1307-12.
10. Dada R, N.P Gupta, K.Kucheiri.Yq microdeletion- Azoospermia Factor Candidate Genes and Spermatogenic Arrest. J Biomol Tech. Sept 2004; 15(3):176-183.
11. Seo JT &Woo JK. Predictive factors of successful testicular sperm recovery in non-obstructive azoospermia patients. Int J Androl. 2001; 24: 306-310.
12. Carpi A, Sabanegh E, Mechanick J. Conteroversies in the management of non obstructive azoospermia. FertilSteril April 2009; 91:963-70
13. Tournaye H .Update on surgical sperm recovery - The European view. THUF 2010 ;(13) 4:242-6
14. McLachlan RI, Rajpert-De Meyts E, Hoei-Hansen CE, de Kretser DM, Skakkebaek NE. Histological evaluation of human testis-approaches to optimizing the clinical value of assessment: Mini Review.2007:22(1); 2-16.
15. Kumar R, Dada R, Gupta NP , Kucheria K. Serum FSH levels and testicular histology in infertile men with non obstructive azoospermia and Y chromosome microdeletions. IJU Dec 2006; 22(4):332-336.
16. World Health Organisation. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th Ed. Cambridge University Press; 1999.
17. Relationship between testicular volume and testicular function: comparison of the Praderorchidometric and ultrasonographic measurements in patients with infertility. Asian J Androl 2008; 10:319–324.
18. Y.Khalifa and J.G.Grudzinskas. Micro-epididymal sperm aspiration or percutaneous epididymal sperm aspiration? The dilemma.Hum Repro1995; 11(3):680-684.
19.I.L.Craft,Y.Khalifa,A.Boulous,M.Pelekanos,C.Foster,M.Tsirigotis. Factors influencing the outcome of in-vitro fertilization with percutaneous aspirated epididymalspermatozoa and intracytoplasmic sperm injection in azoospermic men. Hum Repro 1995; 10(7):1791-1794.
20. Shrivastav P, Nankarni P, Wenswoort S, Craft I: Percutaneous epididymal sperm aspiration for obstructive azoospermia. Hum Reprod 1994;9:2058-2061.
21. Craft I,TsirigotisM.Simplified recovery, preparation and cryopreservation of testicular spermatozoa. Hum Reprod 1995; 10:1623-1627.
22. Hamisu M. Salihu and Muktar H. Aliyu. Sperm retrieval in infertile males: Comparison between testicular sperm extraction and testicular sperm aspiration techniques. Wiener Klinische Wochenschrift 2003; 115(11):370-379.
23. Lisa A. Cerilli, Wayne Kuang, David Rogers. A Practical Approach to Testicular Biopsy Interpretationfor Male Infertility. Arch Pathol Lab Med. 2010; 134:1197–1204.
24. Testis Biopsy and the Infertile Male .Sherman J. Silber. Office Andrology. Contemporary Endocrinology 2005. P.E.Patton, D.E.Bataglia (Eds). Humana Press Inc.Totowa, NJ. Pg 215-240, DOI: 10.1007/978-1-59259-876-2_15.
25. Bruce Ian Bogart andVictoria Ort. Elsevier's Integrated Anatomy and Embryology.ISBN 1416031650 Mosby Published June 2007. pg 669-680.

26.The Developing Human: Clinically Oriented Embryology, 8th Edition. Keith L.Moore&T.V.V.N.Persaud. Elesvier.pg263-265. ISBN: 9781416037064

26. Khatoon F, Ibrahim ,Hafeezullah et al .Association of BMI with Follicular stimulating Hormone and with sperm parameters in males. IOSR Journal of Nursing and Health Science (IOSR-JNHS e-ISSN: 2320–1959.p- ISSN: 2320–1940 Volume 3, Issue 1 Ver. III (Jan. 2014), PP 13-23

27. Khatoon F et al .Correlation and reproductive hormone and fructose with sperm count and motility in infertile males. SAJMR Spectrum: A Journal of Multidisciplinary Research Vol. 3 Issue 5, May 2014

28. Khatoon F, Essaa Abdullah F , Mushtaq M , Balouch Z .Correlation of Fructose with Spermatogenesis. Pinnacle Biochemistry Research.Vol 1(1) 2014.

29. Khatoon F, Alam N, Mahmood A Imtiaz F,. Antisperms antibodies in infertile males attending a Tertiary Care Hospital in Karachi. Pak J Med Sci 2012;28(1):171-174

30. Male Gametogenesis. Textbook of assisted reproduction for scientists in reproductive technology. Steven Fleming and Simon Cooke. Fremantle, W.A :Vivid Publishing, 2009. 1st Ed. Pg 3-11 .ISBN 9780980545913Khatoon F ,Mahmood A .Association Of BMI with Testosterone.Journal of the Dow University of Health Sciences Karachi 2012, Vol. 6 (2): 62-65.

31. Khatoon F at el. Association between BMI and Prolactin Levels in Male Patients Science Journal of Medicine and Clinical Trials. ISSN: 2276-7487 Volume 2013, Article ID sjmct-259, 6 Pages, 2012

32. Aboelnaga SM, F Kahtoon, R Hameed. Association between Serum Creatinine Kinase and Oral Submucousal Fibrosis. Bull. Env. Pharmacol. Life Sci., Vol 9[5] April 2020 : 125-127

33. Aboelnaga SM & Khatoon F. Effect of Mobile phone radiation on human health. Advances in Biotechnology and Microbiology, 16(1) 2020: 138-140. doi:10.19080/AIBM.2020.16.555926- ISSN 2474-7637

34. Alkwai H, Aboelnaga SM, Hussain RA, Kahtoon F. Surfactant protein D levels with obesity and type 2 diabetes mellitus [review article]. Gomal J Med Sci 2020 Jan-Mar;18(1):39-42. https://doi.org/10.46903/ gjms/18.01.2080 jan 2020

35. Khatoon F. Correlation of anti oxidant Zinc with Variousdiet pattern (Fructose oxonicacid ,fructose and oxonic acid) in induced Hyperuricemia. Advances In Bioresearch Vol 10(4) July2019

36. Khatoon F, F Aysha et al. Effect of Lead toxicity and correlation with different variables of DNA damages. JIDMC, journal of Islamabad medical and dental college Vol 7(2) May (2018)

37. Khatoon F , Essa F. Human Genetic Association Studies: association between of Human genome with Clinical outcome. Journal of Biotechnology and Microbiology..Vol 10.(1) 2018 ISSN :2474-7637

38. Khatoon F etal.Polymorphism Of Apolipoprotein B And Its Correlation With Adiposity. Spectrum: A Journal of Multidisciplinary Research Vol. 1 Issue 5, May 2014, ISSN 2278-0637, 180-191
39. Khatoon F etal.Polymorphism Of Apolipoprotein B And Its Correlation With Adiposity. Spectrum: A Journal of Multidisciplinary Research Vol. 3 Issue 5, May 2014, ISSN 2278-0637, 180-191(2014)
40. Khatoon F,Zahid B . Role Of Dietary Copper Supplementation As An Antioxidant In The Induced Hyper Uricemic Rat Model. Journal of international academic research for multidisciplinary, Volume 5, Issue 12, January 2018.ISSN; 2320-5083http://www.jiarm.com/JAN2018.
41. Khatoon F, Farhan Essa Abdullah. Human Genetic Association Studies: association between of Human genome with Clinical outcome. Adv Biotech & Micro. 2018; 10(1): 555778. DOI: 10.19080/AIBM.2018.10.555778
42. HendAlkwai ,Fahmida K. Significance And Role Of Genome Wide Association Studies In relation To Current Scenario Of Human Genomic Framework, Advances In Bioresearch, Volume 9 (6), Nov 2018.ISSN: 0976-4585 DOI: 10.15515/abr.0976-4585.9.6.163170]
43. Fahmida K .Use of Deoxy ribonucleic Acid in human identification. GomalJournal.of Medical Sciences,Volume 16 Oct-Dec 2018 ISSN: 1819-7973 https://www.pakmedinet.com/43017
44. Fahmida K, F Aysha et al. Effect of Lead toxicity and correlation with different variables of DNA damages in azospermia. JIDMC ,2018 (7)2. PG 102-107
45. Wierssema NJ, Drukker AJ, Tien Dung MB, Nhu GH, Nhu NT and Lambalk CB. Consequences of infertility in developing countries: results of a questionarre and interview survey in the south of Vietnam. J Transl Med.2006;4:54.