Original Article

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Seminal fluid analysis changes after testicular varicocelectomy in a sample of Iraqi patients

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ABSTRACT

Background: Semen analysis measures ejaculate volume, pH, sperm count, motility, forward progression, and morphology. Although semen analysis is not a test for infertility, it is considered the most important laboratory test in the evaluation of male fertility. There are many factors affecting the seminal fluid parameters and testicular varicocele is one of them. Varicoceles are the most commonly seen and correctable male infertility factor. Varicocelectomy is a common operation performed for infertile males with clinical varicocele. The aim of study to evaluate the seminal fluid parameters changes after testicular varicocelectomy.

Patients and Method: A prospective cohort study was done at the Al Sader Medical City in Najaf during the period from (March-October 2019). The study include 30 males with age between 18 and 32 years old who had a clinical varicocele. Seminal fluid analysis was done one before surgery and another one three months after surgery and compare between the two tests to evaluate the changes in the parameters was done, which include seminal fluid liquefaction, volume, color, PH, sperm concentration, motility and morphology.

Results: There were 30 patients enrolled in this study with a mean age of 23.8 ± 3.36. Seminal fluid concentration was 19.1 ± 7.2 million/ml and it was significantly increased postoperatively by almost 84% than preoperative concentration to reach 35.1 ± 11.3 ($P \leq 0.001$). The changes in progressive motile sperms' percent improved after varicocelectomy but not reach the statistical significance ($P \geq 0.935$). The mean ± SD percent of normal morphology sperms percent preoperatively was 46.9 ± 22.9 %, and after operation, it elevated to 50.9 ± 18.6 (p value≥ 0.336).The patients is divided in to two subgroups (normospermic and oligospermic subgroup).

Conclusion: There is a significant improvement in sperm concentration. While there is significant improvement in sperm concentration in oligospermic subgroup, so the infertile patient who has oligospermia with varicocele will get better outcome after varicocelectomy.

Keywords: Seminal fluid, analysis changes, testicular varicocelectomy.

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INTRODUCTION

Seminal fluid analysis is the cornerstone in the investigation of male partners of infertile couples; it has been shown to be ineffective in reliably predicting the status of fertility of males. There are an inherent variability in seminal fluid parameters among males [1, 2]. There is general agreement that semen volume and sperm concentration will increase with prolonged sexual abstinence, but at the same time it can affect negatively on motility, viability [3]. Normal semen is gray-white in color and opalescent in appearance. A brown or red hue may indicate the presence of blood, whereas yellow coloration has been associated with certain drugs administration [4,5]. Agglutination, is defined as the sticking together of motile sperm, is evident by microscopic examination of a wet preparation. Although some clumping of immotile sperm may occur in normal seminal specimens, the observation of distinct head-to-head, head-to-tail, or tail-to-tail orientation of sperm is associated with the presence of sperm-agglutinating antibodies [4, 6]. *Hyperspermia*: Volume >5ml and it is associated with the function of male accessory glands. *Normozoospermia*: Sperm count 15 million/ml to 150 million/ml; *Oligozoospermia*: Sperm count below 15 million/ml; *Azoospermia*: Complete absence of spermatozoa in the ejaculation [7]. Viscosity of seminal fluid was reported as normal when the length of the thread did not exceed 2 cm. normally, liquefied semen has low viscosity. Semen that retains some of its viscous properties post-ejaculation, which does not change over time, can be regarded as hyper viscous (SHV) according to the World Health Organization (WHO), diagnosis is based on the thread length formed by liquefied semen [8, 9]. Seminal pH is close to neutral, in the vaginal acid medium provides the spermatozoa the conditions to reach and penetrate the cervical mucus. The ideal pH of human semen has been a matter of discussion so, it is important to note that the pH > 7.2 interpreted literally as normal [10, 11]. Varicocele is defined as dilation of the pampiniform venous plexus. Varicoceles are the most common and treatable cause of men infertility it is believed that it is the cause of up to 35% of primary infertility and 69-81% of secondary infertility [12,13]. The vast majority of adolescent patients with varicoceles are asymptomatic [14, 15] it may be an incidental finding, being discovered accidently an adult patient with a varicocele complains either form a scrotal pain or heaviness at the scrotal sac or form an infertility problems [16, 17]. Repair of varicocele results with different surgical techniques are satisfactory achieving the disappearance of pain of scrotum, if present, and a pregnancy rate about 38 % of patients with fertility problems [18, 19]. The aim of study to evaluate the seminal fluid parameters changes after testicular varicocelectomy.

PATIENTS AND METHOD

Prospective cohort study done at the Al Sader Medical City in AL-Najaf during the period from (March-October 2019). After informed consent. Thirty patients were included in this study. All of them are married men between 18 to 35 years old with the duration of primary or secondary infertility the (term primary infertility is used when a woman has never conceived and secondary infertility is the incapability to conceive in a couple who have had at least one successful conception in the

past) more than one year with varicocele willing to participate were included varicocele willing to participate were included. All of them were diagnosed previously by urologist with testicular varicocele by examination and ultrasound. Collection of samples was conducted in laboratory of Al-Sader Medical City, exclusion criteria: Azoospermia, Patient with subclinical varicocele (grade one), Impaired semen quality, Patient with andrological disease, Patient with chronic systemic disease, any history of testis trauma that might affect the spermatogenesis, patient with torsion, history of mumps or orchitis. Semen samples were obtained by masturbation after 3-5 days of sexual abstinence. Normal semen coagulates (forms clots) a few seconds after ejaculation, and then undergoes liquefaction within 30 minutes manual method for seminal analysis was used. In seminal vesicles by action of the prostatic clotting enzyme coagulation occur because of fibrin clot formation last from 15-30 min. As soon as the semen is liquefied, take the whole semen sample in a tube and seminal fluid, its volume 1.5 – 5 ml with greyish white color. After that put PH stripe in liquefied semen for 45 – 50 sec. and seen the color changes then match the color with the color of stripe provided then note the corresponding PH value normal value 8.0 (alkaline). Prepare the sample to be mobilized sperms by adding diluent such as 3%saline that preserve sperm dilution by factor 400, sperm concentration /ml equals the average of the total count from two counting chambers x5 (to make up the other 20 squares) x dilution factor (usually 400) x 10000 (volume of haemocytometer) normal value: 20-150 million /ml. By using the counter the first one for progressive sperm (rapid) the second for slowly progressive (sluggish) and the third for immotile ones and calculated as percentage normally >40% rapidly motile. On the same slide of motility but microscope, power is 100x. The shape of the sperm seen, the head, middle piece tail and any abnormal morphology can be noticed and calculate them normally: normal shape >60%. Criteria for diagnosis: sperm liquefaction 15-30min, seminal fluid volume 1.5-5ml, sperm ph 8, sperm concentration more than 20-150million cell per ml, sperm motility more than 40% rapidly motile, sperm morphology more than 60% normal shape. The period after operation for seminal fluid collection was 3monthes. Ethical approval was informed consent. Data analysis statistically by SPSS 22 categorical data used frequency and percentage; continuous data used mean and SD. Chi-square test and Fischer exact test used to show association between categorical data. P-value considered significant when equal or less than 0.05.

RESULTS

Prospective cohort study here were 30 patients enrolled in this study with a mean age of 23.8 ± 3.36 (range: 19 – 32) years. The mean ± SD seminal fluid concentration of the 30 patients was 19.1 ± 7.2 million/ml and it was significantly increased postoperatively by almost 84% than preoperative concentration to reach 35.1 ± 11.3 with a mean ± SD difference of 16.0 ± 11.7 million/ml at postoperative, ($P \leq 0.001$, significant). As table (1).

Table (1): Comparison of seminal fluid concentration before and after varicocelectomy of the studied group

Concentration	Mean ± SD	Median	IQR Lower	IQR Upper
Before varicocelectomy	19.1 ± 7.2	12.0	8.0	20.0
After varicocelectomy	35.1 ± 11.3	28.0	12.0	43.0
Mean difference (After - Before)	16.0 ± 11.7	15.5	6.0	24.0
Percentage change	83.9%			
P. value (paired t test)	**0.001***			
Percentage change = value of mean difference / value before varicocelectomy				
***(Significant)**				

Mean ± SD of progressive motile sperms' percent was 39.8 ± 17.1 percent while post operation the mean ± SD was increased to 40.2 ± 19.3 percent, with non-significant different between pre and post operation. Regarding the non-progressive motile sperms' percent, in preoperative seminal fluid analysis was 14.1± 7.1% and 16.0 ± 9.2% post operation with non-significant different between pre and post operation. Immotile sperms not improved after varicocelectomy, and the change was statistically insignificant, preoperative was 42.8 ± 18.5% and at postoperative was relatively higher to reach 43.9 ± 19.3%. As in table (2).

Table (2): Comparison of Progressive motility, Non-progressive and immobility of sperm before and after varicocelectomy of the studied group.

Progressive motility	**Mean ± SD**	**Median**	**IQR Lower**	**IQR Upper**
Before varicocelectomy	39.8 ± 17.1	41.0	30.0	50.0
After varicocelectomy	40.2 ± 19.3	40.0	30.0	54.0
Mean difference (After - Before)	0.4 ± 28.5	-2.0	-15	20
Percentage change	1.1%			
P. value (paired t test)	0.935 *(Not significant)*			
Percentage change = value of mean difference / value before varicocelectomy				

Non-progressive	Mean ± SD	Median	IQR Lower	IQR Upper
Before varicocelectomy	14.1 ± 7.1	12	11	15
After varicocelectomy	16.0 ± 9.2	13.5	12	16
Mean difference (After - Before)	1.9 ± 11.3	-2.0	-3.0	2.0
Percentage change	13.20%			
P. value (paired t test)	0.374 *(Not significant)*			
Percentage change = value of mean difference / value before varicocelectomy				

Immotile	Mean ± SD	Median	IQR Lower	IQR Upper
Before varicocelectomy	42.8 ± 18.5	45	32	50
After varicocelectomy	43.9 ± 19.3	40	30	56
Mean difference (After - Before)	1.1 ± 27.5	0.0	0.0	17
Percentage change	2.50%			
P. value (paired t test)	0.831 *(Not significant)*			
Percentage change = value of mean difference / value before varicocelectomy				

Preoperatively normal morphology sperms was 46.9 ± 22.9 %, after operation 50.9 ± 18.6, this elevation did not reach the statistical significance as in table (3).

Table (3): Comparison of Normal morphology sperms' percent before and after varicocelectomy of the studied group (N = 30)

Normal morphology	Mean ± SD	Median	IQR	IQR
			Lower	Upper
Before varicocelectomy	46.9 ± 22.9	47	35	60
After varicocelectomy	50.9 ± 18.6	54	42	58
Mean difference (After - Before)	4.0 ± 22.3	-1	-12	5
Percentage change	8.50%			
P. value (paired t test)	0.336 *(Not significant)*			
Percentage change = value of mean difference / value before varicocelectomy				

The mean ± SD percent of abnormal morphology sperms percent was 49.8 ± 23.1 %, at preoperative analysis. Postoperative results showed 47.4 ± 18.1%, the reduction was statistically insignificant as in table 4.

Table 4: Comparison of abnormal morphology sperms' percent before and after varicocelectomy of the studied group (N = 30)

Abnormal morphology	Mean ± SD	Median	IQR	IQR
			Lower	Upper
Before varicocelectomy	49.8 ± 23.1	50	40	65
After varicocelectomy	47.4 ± 18.1	45	43	58
Mean difference (After - Before)	-2.4 ± 18.7	1	-5	12
Percentage change	-4.70%			
P. value (paired t test)	0.496 *(Not significant)*			
Percentage change = value of mean difference / value before , negative sign indicates a reduction after varicocelectomy,				

Further analysis was performed to assess the correlation between the studied parameters, the correlation matrix is shown in fig. (1-8), 10 significant correlations were found as followed;

1. Inverse (-ve) significant correlation between normal and abnormal morphology percent (r = -0.938, P≤0.001).
2. Direct (+ve) significant correlation between abnormal morphology and immotility (r = 0.808, P≤0.001)
3. Inverse (-ve) significant correlation between normal morphology and immotility (r = -0.818 , P≤0.001)
4. Inverse (-ve) significant correlation between abnormal morphology and non-progressive motility (r = -0.456, P ≤ 0.011)
5. Inverse (-ve) significant correlation between abnormal morphology and progressive motility (r = -0.594, P≤0.001)
6. Direct (+ve) significant correlation between normal morphology and progressive motility (r = 0.674, P ≤ 0.001)
7. Inverse (-ve) significant correlation between immotility and progressive motility (r = -0.886, P≤0.001)
8. Direct (+ve) significant correlation between concentration and non-progressive motility (r = 0.365, P ≤ 0.047).

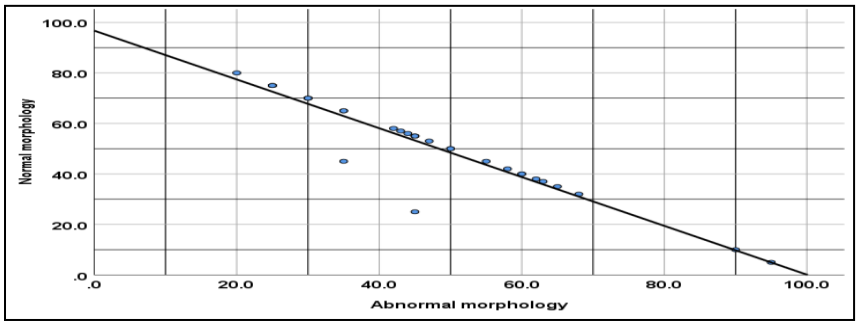

Figure (1): Curve estimation diagram showing the significant inverse (negative) correlation between normal and abnormal morphology percent r =-0.938

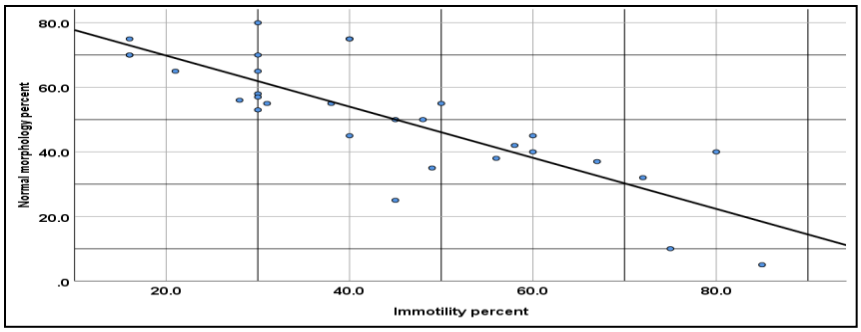

Figure (2): Curve estimation diagram showing the significant inverse (negative) correlation between Normal morphology and immotility r=-0.818

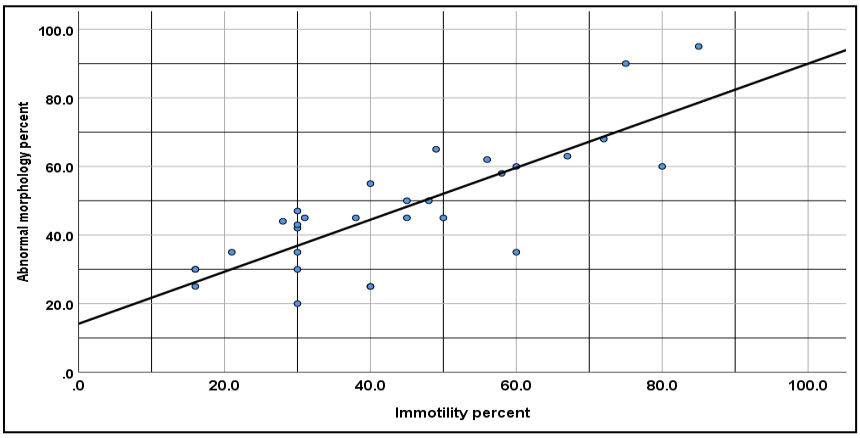

Figure (3): Curve estimation diagram showing the significant direct (positive) correlation between abnormal morphology and immotility=0.808.

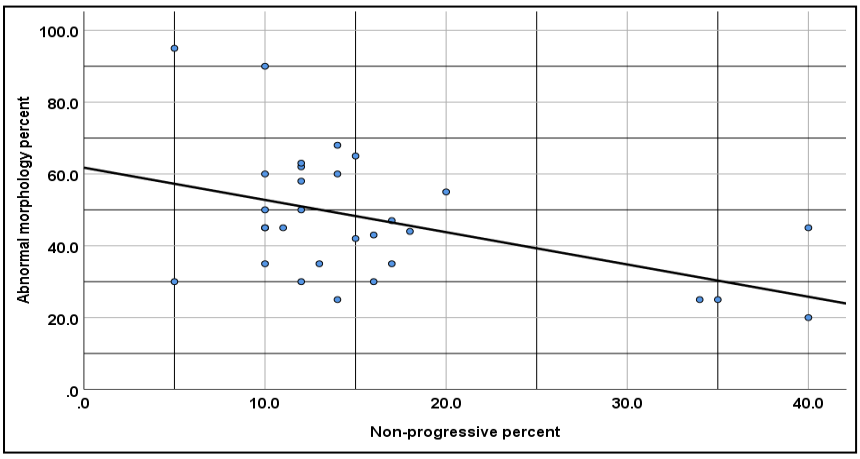

Figure (4): Curve estimation diagram showing the significant inverse (negative) correlation between abnormal morphology and non-progressive motility r= -0.456

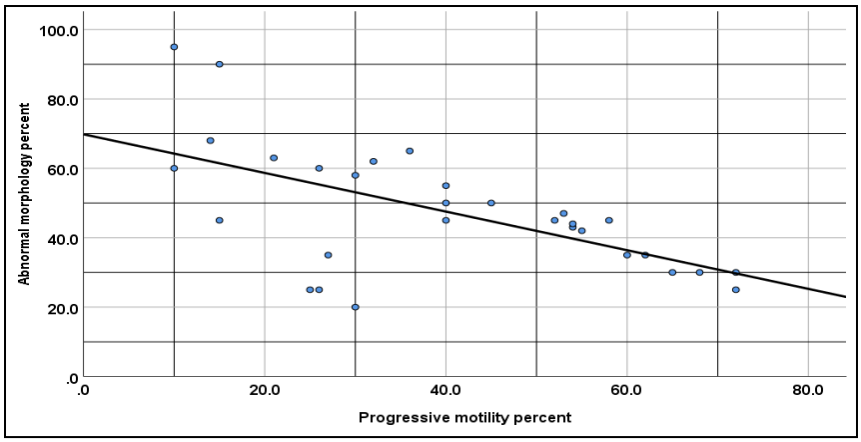

Figure (5): Curve estimation diagram showing the significant inverse (negative) correlation between abnormal morphology and progressive motility r= -0.594

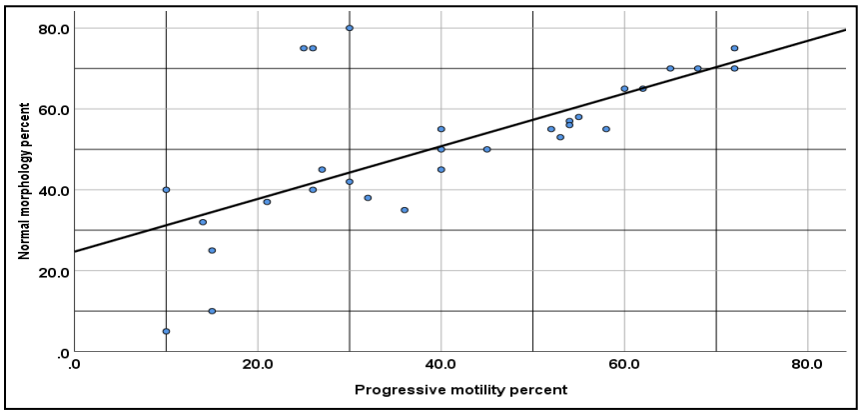

Figure (6): Curve estimation diagram showing the significant direct (positive) correlation between normal morphology and progressive motility r= 0.674

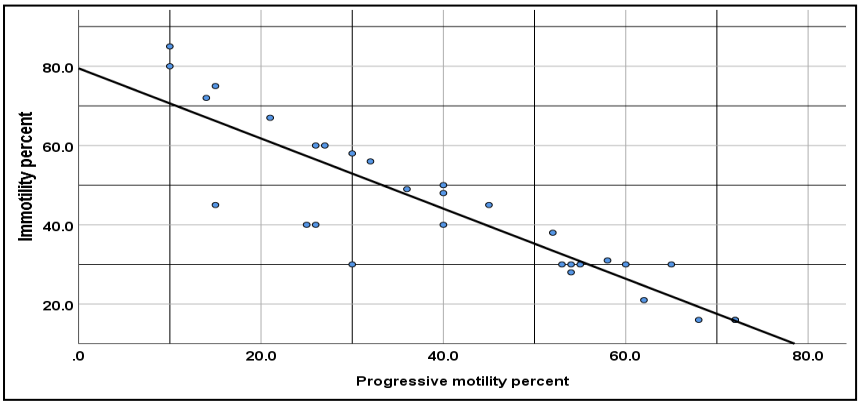

Figure (7): Curve estimation diagram showing the significant inverse (negative) correlation between immotility and progressive motility r= -0.886

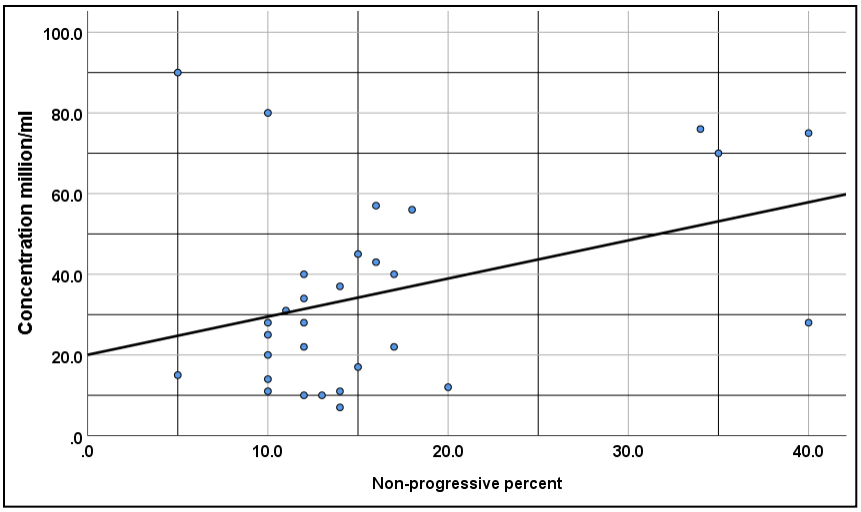

Figure (8): Curve estimation diagram showing the significant direct (positive) correlation between concentration and non-progressive motility r= 0.365.

DISCUSSION

Testicular Varicoceles are the most commonly seen correctable male infertility factor, occurs in approximately 15% of male in the general population. The prevalence of varicocele increases to 35% of males with primary infertility and between 75% and 81% of males with secondary infertility [(20)].

The present study found that the liquefaction time mean was 33.9±3.3 minutes before varicocelectomy and 32.6 ±4.8 minutes three months after varicocelectomy, which is not significant. The volume of ejaculate in the current study, there is only 5.4% change in postoperative semen volume and statistically not significant. Mohamed *et al* study showed no significant change with postoperative semen volume that agree with the current study [(21)]. In current study the mean concentration of sperm before varicocelectomy was 19.1±7.22, while three months after varicocelectomy the mean of sperm concentration was 35.1±11.27 (increased by 84%) with a very significant. Ghanaie *et al*. iranian study showed a significant improvement in sperm concentration by allmost 75% and $P \leq 0.001$[(22)]. Another Iranian study was conducted by Abdel-Meguid *et al*. study supported the finding of Ghanaie et al study were a significant improvement in sperm concentration had been reported in varicocelectomy group [(23)]. Regarding the comparison between values of motility subtypes in preoperative and postoperative period , after varicocelectomy the present study found the difference did not reach the statistical significance and progressive motility percent, Ghanaie *et al*. agreed with our study which shows mild improvement in sperm motility after 3 months of their study while show significant improvement after 6 month of follow up after varicocelectomy [(22)].

The effect on morphology In normospermic subgroup no improvement In oligospermic subgroup there was better out come after operation significant or not. Ghanaie *et al.* study which showed little improvement in 1st three months after surgery while good improvement after more follow up for their patient [22]. The patient who presented with normospermia even with lower limit of normal value show no significance and sometimes worsening in sperm concentration three months after varicocelectomy that agree with Okeke *et al* study that show no significant improvement in sperm concentration [23]. Also in normospermia subgroup the liquefaction has no significant change that can be due to their liquefaction is within normal value preoperatively. The volume of ejaculate in normospermic patient had no significant change that agree with Okeke *et al* study that show, no significant improvements in semen volume. There was no improvement in motility in normospermic patient that was agree with Okeke *et al* Study with sperm motility. In the current study in normospermic subgroup there was no significant improvement in sperm morphology after varicocelectomy that is agree with with Okeke et al study that reveal no significant improvement in sperm morphology after varicocelectomy [24]. In oligospermic subgroup who undergone varicocelectomy there was better outcome in the sperm concentration which show significant improvement that was agree with Gupta *et al* study which involved 56 patients with clinical varicocele and oligoasthenospermia that show significant improvement in the mean sperm concentration [25].

In the current study the motility have not significant statistical improvement which disagree with Gupta *et al* Study and disagree with Anyadike *et al* Study [26]. Also in the current study, there is no significant improvement in spermatic fluid volume that disagree with Anyadike CC *et al* which is significant statistically [26]. Also regarding oligospermic subgroup, the sperm morphology show no significant improvement that is agree with Anyadike *et al* study and disagree with Gupta *et al* study [25]. Furthermore as a secondary outcome of the current study further analyses were performed to assess the correlation between seminal fluid parameters, these analyses revealed a negative significant correlation between normal and abnormal morphology with a (P value ≤ 0.001) and this result was a common sense as summation of both factor form 100%.

CONCLUSION

There is a significant improvement in sperm concentration with no significant improvement in motility, also no significant improvement in sperm morphology. In normospermic subgroup there is no significant improvement in all parameters, While there is significant improvement in sperm concentration in oligospermic subgroup, so the infertile patient who has oligospermia with varicocele will get better outcome after varicocelectomy.

REFERENCES:

1. Wang C, Swerdloff RS. : Limitations of semen analysis as a test of male fertility and anticipated needs from newer tests. Fertility and Sterility. 2014 Dec 1;102(6):1502-7.

2. De Jonge C, LaFromboise M, Bosmans E, Ombelet W, Cox A, Nijs M.: Influence of the abstinence period on human sperm quality. Fertility and sterility. 2004 Jul 1;82(1):57-65 .

3. Goyena, R. & Fallis, A. G. Fundamentals of Urine & Body Fluid Analysis. Journal of Chemical Information and Modeling 53, 1689–1699.(2019)

4. Baliyan A, Dhingra H, Tahlan A: Significance of Spe rm Characteristics in the Evaluation of Male Infertility in a Tertiary Care Centre. Annals of Pathology and Laboratory Medicine. 2017 Oct 27;4(5):A536-540.

5. Rothmann, S. A., Bort, A. M., Quigley, J. & Pillow, R. Sperm morphology classification: A rational method for schemes adopted by the world health organization. Methods in Molecular Biology 927, 27–37.(2013)

6. Meacham R.: Perspectives and Editorials. Journal of Andrology. 2002 May 6;23(3):330-1.

7. Ye T, Liu J, Li Y, Xue S, inventors; Ahead Biotech Co Ltd, assignee.: Quantificational testing method for semen liquefaction ability. United States patent application US 15/510,433. 2017 Sep 14.

8. Choi WS, Kim SW. :Current issues in varicocele management: a review. The world journal of men's health. 2013 Apr 1;31(1):12-20.

9. Greenfield SP, Seville P, Wan J.: Experience with varicoceles in children and young adults. The Journal of urology. 2002 Oct;168(4 Part 2):1684-8.

10. Naughton CK, Nangia AK, Agarwal A.: Varicocele and male infertility: part II: pathophysiology of varicoceles in male infertility. Human reproduction update. 2001 Sep 1;7(5):473-81.

11. Esteves SC, Agarwal A. :Afterword to varicocele and male infertility: current concepts and future perspectives. Asian journal of andrology. 2016 Mar;18(2):319.

12. Wein, A. J., Kavoussi, L. R., Novick, A. C., Partin, A. W. & Peters, C. a. Campbell-Walsh Urology 10th Edition. Campbell-Walsh Urology 1834–1846 (Elsevier Inc., 2012).

13. Mohamed EE, Gawish M, Mohamed A. Semen parameters and pregnancy rates after microsurgical varicocelectomy in primary versus secondary infertile men. Human Fertility. 2017 Oct 2;20(4):293-6.

14. Ghanaie MM, Asgari SA, Dadrass N, Allahkhah A, Iran-Pour E, Safarinejad MR. Effects of varicocele repair on spontaneous first trimester miscarriage: a randomized clinical trial. Urology journal. 2012 May 24;9(2):505-13.

15. Abdel-Meguid TA, Al-Sayyad A, Tayib A, Farsi HM. Does varicocele repair improve male infertility? An evidence-based perspective from a randomized, controlled trial. European urology. 2011 Mar 1;59(3):455-61.

16. Okeke L, Ikuerowo O, Chiekwe I, Etukakpan B, Shittu O, Olapade-Olaopa O. Is varicocelectomy indicated in subfertile men with clinical varicoceles who have asthenospermia or teratospermia and normal sperm density?. International Journal of Urology. 2007 Aug;14(8):729-32.

17. Gupta C, Chinchole A, Shah R, Pathak H, Talreja D, Kayal A. Microscopic varicocelectomy as a treatment option for patients with severe oligospermia. Investigative and clinical urology. 2018 May 1;59(3):182-6.

18. Anyadike CC, Ekeke ON, Eke N. Effect of varicocoelectomy on seminal fluid parameters. Journal of the West African College