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RESEARCH ARTICLE

The influence of Leukocytospermia and Teratozoospermia in IUI outcome

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Abstract

Background: Male urogenital tract infection is one of the most important causes of male infertility worldwide. The presence of leukocytes in the semen has been associated with poor semen quality and positive correlation between leukocytospermia and abnormal sperm morphology.

Objective: This investigation will be analyzed the outcome of the intra uterine insemination (IUI) cycles in which the male partner exhibited leukocytospermia and/or teratozoospermia.

Subjects, Materials and methods: This study includes seventy men their ages between 20- 45 years old who attended to High Institute of Infertility Diagnosis and Assisted Reproductive Technology / AL-Nahrain University and Al-Imamein Kadhymia Medical City at Um Al- Banein Center .This prospective study was accomplished through the period from November 2014 till March 2015.

Results:

The study found that sperm concentration in the forth groups were significantly ($P<0.01$) lower after *in vitro* sperm activation(ISA) compared with before ISA. The active Sperm motility a+b and morphologically sperm normality were reached the highly significant ($p<0.01$) increase after ISA compared with before ISA. The number of round cells after ISA was a significantly ($P<0.01$) decreased compared to before ISA, the mean of leukocytes by Endtz test method after ISA was significantly ($P<0.01$) reduced compared with before ISA. The total pregnancy rate after ISA was (12.8%) per cycle after *in vitro* activation technique and IUI, the pregnancy rates following IUI in normozoospermic group (20%) ,leukocytospermic group (13.3%), teratozospermic group(6.6%) and leukocytospermic-teratozospermic group (6.6%).

Conclusions: The pregnant rate after IUI in leukocytospermic and teratozoospermic couples was low compared to normozoospermic couples. Thus the infertile men with abnormal sperm form and high number of leukocytes are recommended to ensure the sperm normality following *in vitro* activation techniques by Kruger criteria and peroxidase (ENDTZ) test with cytogenetic study before intra uterine insemination**.**

INTRODUCTION

Male urogenital tract infection is one of the most important causes of male infertility worldwide. It has been reported that genital tract infection and inflammation have been associated with 8-35% of male infertility cases [1].

Leukocytospermia is thought to have multifactorial origin. In addition to genital tract infections, other etiologies such as smoking, alcohol consumption[2] Increased WBC in semen can be detected in men with abnormal spermatogenesis as protective mechanism for the removal of defective sperm from the ejaculate [3]
Leukocytes are a frequent finding in seminal plasma of infertile male patients, even in the absence of inflammatory symptoms. In many cases, leukocyte concentrations in seminal fluid are a pathological level according to the WHO (1999) criteria. The frequency of leukocytospermia among infertile males is 30%, and in 80% of leukocytospermic patients, no microbial infection can be detected in seminal fluid. Therefore, the correlation between leukocytospermia and male infertility is controversial when some studies have failed to find any association [4,5]
The presence of leukocytes in the semen has been associated with poor semen quality and positive correlation between leukocytospermia and abnormal sperm morphology[6,7].
The shape of the sperm is a reflection of sperm development in the testicle, or spermatogenesis. Men with a defect in sperm maturation tend to have problems with sperm morphology and may then be at risk for failure of their sperm to fertilize their partner's eggs [8].
On the other hand, the morphologic characteristics of the sperm cell are the outcome of highly complex cellular modifications occurring during spermatogenesis [9,10,11]. The resulting percentage of abnormal spermatozoa as well as specific structural abnormalities may serve as an indicator of a defective mechanism related to spermatozoa production and/or maturation [12],and is a valuable predictor of spontaneous pregnancies and fertilization success in assisted reproductive technology (ART) [13,14] . Furthermore, abnormal spermatozoa morphology has been linked not only to a decrease of traditional parameters of semen quality [15,16], but also to an increase in contemporary markers of sperm damage, such as DNA fragmentation [12,17] or reactive oxygen species (ROS) overproduction [18,19]. Thus, morphological evaluation of spermatozoa is an integral part of the semen analysis work-up for infertility. In a meta-analysis of outcomes in intrauterine insemination (IUI) cycles, a trend toward better pregnancy rates was indicated when sperm morphology was >4% [20]. Severe teratozoospermia with <4% normal forms in the semen sample was associated with poorer fertilization outcomes during IVF [21,22].

Material and Methods:

This study includes seventy men their ages between 20- 45 years old who attended to High Institute of Infertility Diagnosis and Assisted Reproductive Technology / AL-Nahrain University and Al-Imamein kadhymia medical city at Um Al- Banein center .This prospective study was accomplished through the period from November 2014 till March 2015. Those patients were classified into subgroups according to WHO criteria of seminal fluid analysis:
1-Normozoospermic group:-Twenty five Males were included in this group.
2-Leukocytospermic group:-fifteen patients were included in this group
with round cells <5/HPF.(WHO,2010)
3- Teratozoospermic group:- fifteen patients were included in this group
morphologically normal sperm< 30%.(WHO,1999)
4- Teratozoospermic and leukocytospermic group:- fifteen patients were included in this group with round cells <5/HPF and morphologically normal sperm< 30%.(WHO,2010,1999)

Microscopic Examination

Microscopic examinations of semen include the following characteristics: sperm concentration, motility, morphology, cells other than spermatozoa, agglutination.

A-Sperm Concentration

Sperm concentration was measured from the mean number of sperm high power fields under magnification of 400 X. This number was multiplied by factor of one million. The total sperm count was obtained by multiplying sperm concentration with semen volume. The lower reference limit for sperm concentration is 15×10^6 spermatozoa per ml and for total sperm number is 39×10^6 spermatozoa per ejaculate**.**

B- Sperm Motility Percent and Grade Activity

One drop (10 µl) of liquefied mixed semen was mounted between slides and covered. Then the slide was examined after waiting for a few seconds to get rid of current, 200 spermatozoa in a total of at least 5 fields was counted and categorized in compliance to WHO simple system in grading motility for 2010, categorization was recorded as follows:
The lower reference limit for total motility (Progressive motility + Non-progressive motility) is 40% and for progressive motility (PR) is 32%

C- Morphologically Normal Sperm Percentage

The examination of morphologically abnormal sperms was performed using the same prepared slides for sperm motility .At least 200 sperm were counted and percentage of morphologically abnormal sperms was calculated from the following formula:

Morphologically abnormal sperms= $\frac{No\ of\ abnormal\ \ sperms}{Total\ \ No\ .of\ sperms}$

Normal value should be equal or more than 30% normal morphology [32].Sperm morphology was performed in several methods, in this study used:

-Kruger Strict Criteria (KSC)

Sperm morphology determined by the Kruger strict morphology method . In fact, the Kruger method is the only morphology scoring system shown to have a clinical predictive value for the sperm fertilizing an egg in IVF. Kruger strict morphology testing can also be completed at the time of intrauterine insemination (IUI).Sperm were judged as normal based on the following criteria:-

Head must be oval in shape with smooth contours, 5-6 µm in length and 2.5 to 3.5 µm wide with the acrosome taking up 40-70% of the head**,** neck and mid-piece must have no abnormalities and a cytoplasmic droplet (a remnant from sperm production) if present must be larger than half the size of the head, tail must not be coiled or bent and should not have a droplet at the end.

Testsimplet stain for Kruger Strict Criteria:

Especial pre-stained slides were used for examination of sperm morphology, the stain contains two dyes: Crystal violet acetate and New Methylene Blue.

One drop (10µl) of semen was mounted between warmed pre-stained slide, covered with standard cover slip and left for 3 minutes at room temperature then examined under light microscope using oil-emersion power 1000X and graduated optic. Ten fields or 100 sperms were examined by measuring the length and width of the head axis for each sperm in the slide by the graduated optic. The normal head axis length is 5-6 µm and normal head axis width 2.5-3.5 µm.

Round Cells: Round cells in semen can be distinguished into:

a) White blood cells b) germinal cells and others.

Differentiation between these cell types is considered of utmost importance for diagnostic and therapeutic purposes[24].In fertile individuals ,round cells usually represent less than 5% (< 5 cells/HPF) of the total cellular content of semen [25].The objective of this study is to differentiate between WBCs and other round cells using peroxidase test .

Endtz (peroxidase) test: The preparation of materials which including :-

A- Preparation of stock solution.

The peroxidase method used was adapted from Endtz[26].A stock solution was prepared by mixing: 50 ml distilled water, 50 ml 96% ethanol, 125 mg benzidine (Sigma Chemical Co.,St. Louis, MO).

B-Preparation of working solution:-

1- Four ml of stock solution was mixed with 5μL of 30% H_2O_2 in a 10 ml test tube.
2- Fresh working solution from stock was prepared every week

Procedure for Endtz :-

A volume of 20µl of liquefied semen specimen was aspirated in a micro-centrifuge tube; then 20µL of working solution was added and mixed thoroughly and incubated for 5 minutes at room temperature. 20µL of this solution were diluted by mixing 20µL of phosphate- buffered saline immediately before counting, neubaur counting chamber was loaded with 5µl of the prepared solution, examination was done under 40 x bright field objective lens, the stained dark brown in color cells with round shape were considered leukocytes, counting of the cells in all RBC fields of Neubaur counting chamber was done, which equal to 1 mm^3, number of WBC was calculated by multiplying total number of cells by 4 to correct it for dilution factor. The total WBC number will be $Nx10^5$/ml semen. This number should be corrected to million/ml by dividing it by 10.

***In vitro* sperm activation**

Simple Layer Technique: Two ml of human semen sample was layered beneath two ml of culture medium. Following 10 and 30 minutes of incubation at 37ºC, a drop was taken from the top of upper layer by micro-automatic pipette to were measured again following the activation [27].

Swim-up Technique : It is based on spermatozoa self-propelled active movement from a single centrifuged, pre-washed cell pellet, into an overlaying medium which serves as a hospitable environment for healthy sperm. Normal spermatozoa move away from seminal plasma, but those with tail abnormalities are not capable of migrating into the

swim-up medium. Only a small fraction of total motile sperm is recovered by the SU methodology, therefore its use is mostly restricted to ejaculates with high sperm counts and good motility [28].

Ovulation induction protocols:

In this study the protocols used for ovulation induction were:

1-Induction of ovulation by clomiphene citrate alone at a dose of 50mg twice daily from day two or three of menstrual cycle for 5 days only [29].

2-Induction of ovulation by Gonal-F injections 75 IU of r-FSH /vial alone in a dose of 75mg once daily for 2 to 10days starting from day 2 or 3 of the cycle or in combination with clomiphene citrate at the same dose. Gonal F is given subcutaneously only [30].

3-Induction of ovulation by Puregon injections 50IU of r-FSH / vial alone in a dose of 50mg once daily for 2 to 10 days starting from day 2 or 3 of the cycle or in combination with clomiphene citrate at the same dose. It is given subcutaneously or intra-muscularly [31].

4- Induction of ovulation by Menogon injections75 IU of both urinary FSH and LH alone in a dose of 50mg once daily for 2 to 10 days starting from day 2 or 3 of the cycle or in combination with clomiphene citrate at the same dose given intramuscularly only [32].

5- Ovitrelle injections 6500IU/vial of Human chorionic gonadotrophin according to Body Mass Index given subcutaneously only to enhance ovulation [33].

6-The choice of stimulation protocol was individual and was based on the patient's age, diagnosis, reproductive history and ovarian response, and coexisting medical conditions.

Vaginal ultrasonographies were performed after induction of ovulation on day (12-14) of the cycle (average cycle day 13) and at least one mature Graffian follicle was present and size of the GF was between (18-24) mm. Then Oviterlle injections 6500IU /vial of human chorionic gonadotrophin (hCG) were given subcutaneously. IUI performed 36-40hours after hCG injection and after the semen of the male partner was prepared by swim-up activation technique.

Intra – uterine insemination (IUI) technique

The wife was prepared for IUI and allowed to be in lithotomic position. A non lubricated Cusco´ s speculum was inserted inside the vagina to visualize the uterine cervix [34]. A special intrauterine catheter was loaded with 0.5-0.7ml of prepared semen and introduced inside the uterus till it reached uterine fundus where the washed semen was pushed slowly and steadily. The spouse was waiting in the insemination room for about 30 minutes lying down. Following 14 days from insemination .

Results

Seventy infertile couples were involved in this study. The mean age of infertile males was 30.05 years with range from 20 to 41 years. However, 57.2% of total couples were suffering from primary infertility, while 42.8% of them got secondary infertility.

Comparison of certain sperm function parameters in the semen of the four groups involving in this study before and after *in vitro* activation:

Figure (1) shown: The sperm concentration in normozoospermic men group before and after *in vitro* activation. The sperm concentration after ISA was significantly ($P<0.01$) lower (32.4$\pm$1.828 million/ ml) than that of before ISA (61.1$\pm$3.297 million/ ml).And The sperm concentration in leukocytospermic men group after ISA was significantly ($P<0.01$) lower (30.4$\pm$1.759 million/ ml) than that of before ISA (57.2$\pm$4.038 million/ ml).While the sperm concentration was significantly ($P<0.01$) in teratozoospermic men group decrease after ISA (26$\pm$1.511million/ ml) than that of before ISA (43$\pm$2.038million/ ml). The mean of sperm concentration in teratozoospermic and leukocytospermic men group after ISA was significantly ($P<0.01$) decrease after ISA (30$\pm$2.007million/ ml) compared to before ISA (44.6$\pm$3.154million/ ml).

Figure (2) shown: The active sperm motility (a+b) in normozoospermic men group before and after *in vitro* activation. The active sperm motility a+ b (75$\pm$2.857 %) after ISA was significantly ($P<0.01$) higher than that of before ISA (46.5$\pm$3.279%). There was a highly significant ($P<0.01$) increase in sperm motility of leukocytospermic men group after ISA (69.4$\pm$2.130%) compared with before ISA (39.6$\pm$1.585%). A highly significant ($P<0.01$) elevation in sperm motility of teratozoospermic men group was observed after ISA (61$\pm$2.179%) compared with before ISA (38.8$\pm$1.844%). The active sperm motility in teratozoospermic and

leukocytospermic men group was significantly (P<0.01) increase after ISA (68±2.476 %) compared with before ISA (35±1.608%).

Figure (3) shown: The percentages of the sperm morphology in normozoospermic men after ISA was (63.4±2.247%) .There was a significant (P<0.01) improvement compared with before ISA (41.3±1.749%).A significant (P<0.01) increase was recorded in leukocytospermic men after ISA (60.0±2.678%) compared with before ISA (39.6±2.961%). The percentage of sperm morphology in teratozoospermic men after ISA was (56.6±3.09%) significantly (P<0.01) higher than that of before ISA (25±0.779%).In teratozoospermic and leukocytospermic men the percentage of sperm morphology (53.6±3.003%) after ISA was significantly (P<0.01) higher than that of before ISA (24.5±1.003%).

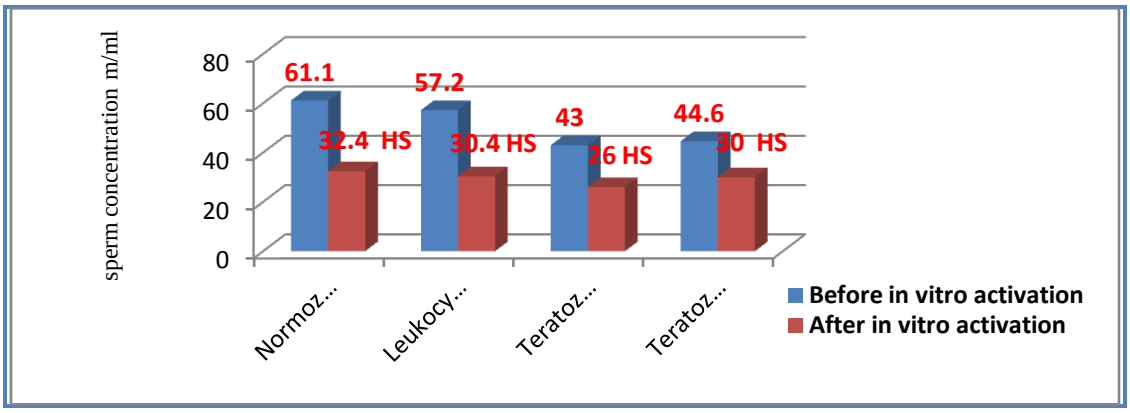

Figure (1): Comparison of the sperm concentration before and after *in vitro* activation between the four groups involved in this study.

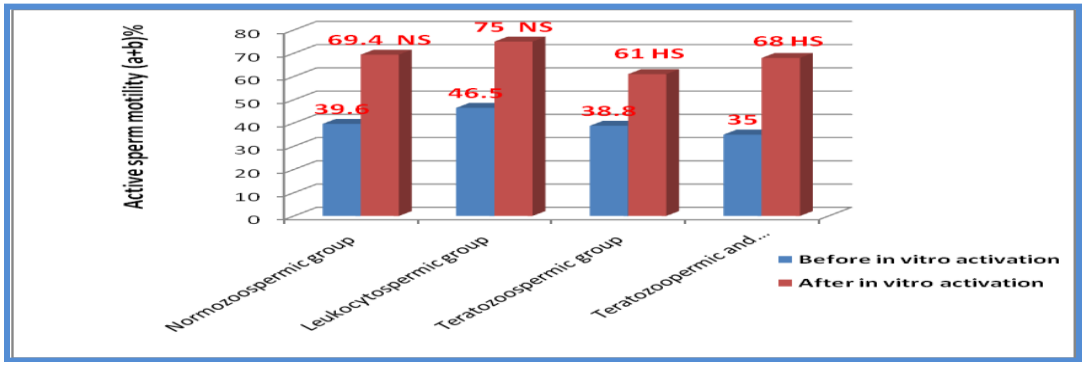

Figure (2): Comparison of the active sperm motility before and after *in vitro* activation between of the four groups involved in this study

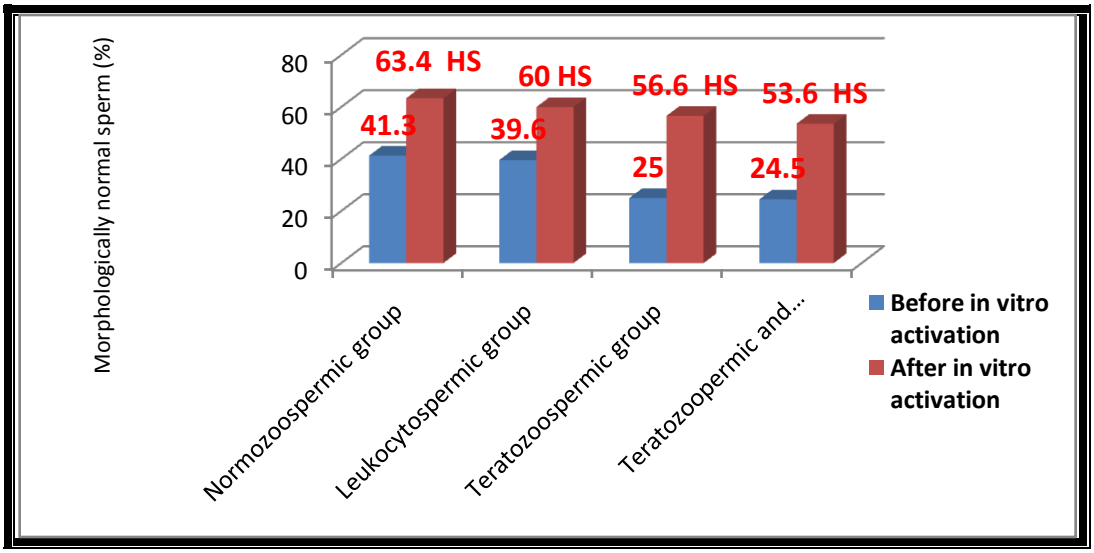

Figure (3): Comparison of the sperm morphology before and after *in vitro* activation between of the four groups involved in this study

Pregnancy rate after IUI for the men groups involved in this study:

The total pregnancy rate in this study was (12.8%) per cycle after *in vitro* activation technique and IUI. As shown in figure (4) pregnancy rates following IUI in group (1) ,(2), (3) and (4), were (20%), (13.3%), (6.6%) and (6.6%) respectively. Those was a significant different between normozoospermic group and Leukocytospermic group , Teratozoospermic group ,Leukocytospermic-Teratozoospermic group (p=0.012,p=0.047,p=0.047 respectively).

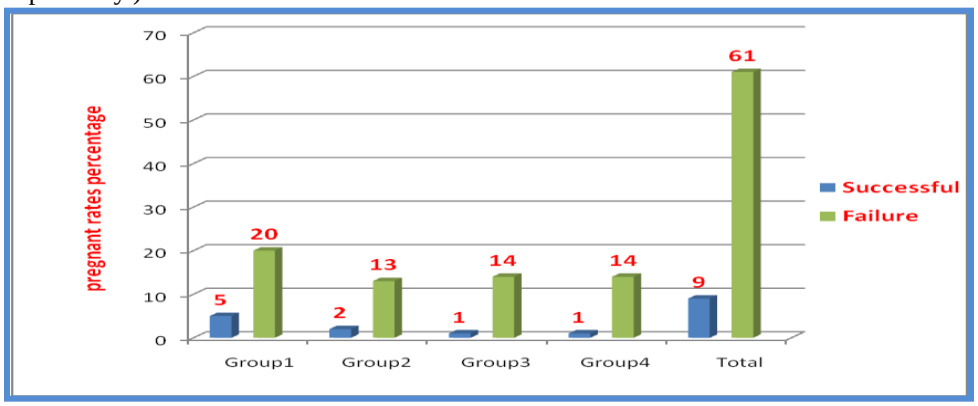

Figure (4): Pregnancy rate in the present study *P<0.01 High significant
Group1: Normozoospermic men
Group2: Leukocytospermic men p=0.012 Significant
Group3: Teratozoospermic men p=0.047 Significant
Group4: Leukocytospermic and Teratozoospermic men p=0.047 p=0.047 Significant

Comparison of morphologically normal sperm by Kruger strict criteria before and after *in vitro* activation between of the four groups

As shown in table (1), the morphologically normal sperm by Kruger strict criteria before and after *in vitro* activation between the four groups. The correlation between normozoospermic men group (5.4±0.108,3.0±0.06) and all patients groups(5.3±0.1,3±0.06),(5.2±0.104,2.9±0.058),(5.1±0.102,2.9±0.058)respectively, was significantly (P<0.05) different in the Kruger strict criteria for the length and width of the sperm head. While the correlation between Normozoospermic men group (5.4±0.108, 3.0 ±0.06) and Leukocytospermic men group (5.3±0.1, 3±0.06) was not significantly(P>0.05) different regarding the length and width of sperm head.

Also in this study found the correlation between Normozoospermic men Group (5.4±0.108, 3.0 ±0.06) and Teratozoospermic group ,Leukocytospermic group (5.2±0.104, 2.9±0.058),(5.1±0.102, 2.9±0.058) respectivly was significantly (P<0.05) different by the Kruger strict criteria when measuring the length and width of the sperm head.

Table (1): Comparison of morphologically normal sperm by Kruger strict criteria before and after *in vitro* activation between of the four groups.

Patients Groups	morphologically normal sperm by Kruger strict criteria	
	Before *in vitro* activation	After *in vitro* activation
Normozoospermic men	L 5.1±0.102 W 2.8±0.056	5.4±0.108 3.0 ±0.06
Leukocytospermic men	L 5.0±0.1 W 2.8±0.056	5.3±0.1 3±0.06 NS
Teratozoospermic men	L 4.7±0.094+ W 2.6±0.052	5.2±0.104* 2.9±0.058*
Leukocytospermic and teratozoospermic men	L 4.5±0.09+ W2.6±0.052	5.1±0.102* 2.9±0.058*

ANOVA of Normozoospermic men with all Patients Groups (L)
F-test 7.811 p=0.031 P<0.05 Significant

- ANOVA of Normozoospermic men with all Patients Groups (W)
F-test 15.001 p=0.008 P<0.05 Significant

*P<0.05 Significant between teratozoospermic and leukocytospermic - teratozoospermic group with normozoospermic after activation

+ P<0.05 Significant between teratozoospermic and leukocytospermic - teratozoospermic group with normozoospermic before activation

Comparison of the number of leukocytes by Endtz test before and after *in vitro* activation between the four groups involved in this study.

Table (2) shown the comparison of leukocytes by Endtz test between Normozoospermic men group and all patient groups(Leukocytospermic, teratozoospermic, Leukocytospermic-teratozoospermic groups).There was a significant (P<0.05) decline after *in vitro* activation compared with before *in vitro activation.*

Table (2) : Comparison of the number of leukocytes by Endtz test before and after *in vitro* activation between the four groups involved in this study.

Patients Groups	Number of Leukocytes detected by Endtz test	
	Before *in vitro* activation	After *in vitro* activation
Normozoospermic men	0.16±0.056	0±0.00 *HS r=0.975
Leukocytospermic men	1.44±0.103	0±0.0 *HS r=0.974
Teratozoospermic men	0.2±0.078	0±0.00 *HS r=0.977
Leukocytospermic and teratozoospermic men	1.49±0.074	0±0.00 *HS r=0.980

ANOVA of Normozoospermic men with all Patients Groups , F-test 4.91 p=0.048 P<0.05 Significant
ANOVA of Normozoospermic men with all Patients Groups without before *in vitro activation p>0.05Non significant*

Discussion

The results of all semen sample groups namely (normozoospermia, leukocytospermia, teratozoospermia, leukocytospermia - teratozoospermia) revealed that there is a high significant decreased (p<0.01) in the sperm

concentration following *in vitro* activation by using (Ham's F-12) medium. This finding due to inability of dead and morphologically abnormal sperm with poor motility to swim up and migrate into upper layer of culture media that used in this work. These results were in agree with other studies (Al-Dujaily and Safaa Meran 2014; Andersen *et al*, 1995;Kouty ,2007; Rasheed ,2012).

Also the data of the present work found a high significant increase ($p<0.01$) in the percentage of active sperm motility grades (A&B) in normozoospermic group, leukocytospermic group, teratozoospermic group and leukocytospermic - teratozoospermic group. A study show this is regarded as normal response for sperm activity after removal of seminal plasma since it containing dead sperm, leukocytes, epithelial cells, particulate debris and microbial contamination that produce many oxygen radicals that can negatively influence the sperm functions as the active sperm motility. Prolonged contact of spermatozoa with seminal plasma may be impact negatively on sperm activity and fertilizing ability [39].

Another factor increase sperm motility is the effects of culture medium Hams F-12. The Hams F -12 medium provides the necessary salts, amino acids and vitamins which required for triggering of intact sperms leading to activation of sperms movement [40]. All these minerals stimulate sperm motility and grade activity of forward movement [41]. This results was agree with (Al-Dujaily *et al*, 2013),(Al-Dujaily and Malik, 2013).

Further high significant improvement ($p<0.01$) in the percentage of morphologically normal sperm was recorded after activation. This finding may be related to the fast movement of normal spermatozoa from seminal plasma into upper layer of culture medium, and consequently may be from impact of some seminal plasma components like leukocytes, round cell and others leading to keep the sperm from the stress factors and ROS production .This results was agree with (Al-Dujaily *et al*, 2013).

There was a highly significant decrease ($p<0.01$) in the round cells after *in vitro* activation compared to before activation. The decrease in number of round cells may be due to semen preparation techniques for assisted reproduction that remove the defective and non vital sperms as well as cells e.g. spermatogenic cells and leukocytes. Leucocytes, bacteria and dead spermatozoa produce oxygen radicals that negatively influence the ability to fertilize the egg [44]. This results was agreement with (Al-Dujaily *et al*, 2013),(Al-Dujaily and Malik, 2013).

In the current research a high significant reduction ($P<0.01$) was noticed in the leukocyte concentration after *in vitro* activation in subject groups, this is because the sperm preparation techniques for ART have been developed to remove the undesired sperm, round cells, debris, and thereby increase the overall sperm quality.

This results was agree with Gambera study (2007). Thus different studies have been used different techniques and shows significant reduction in the count of round cells [46,47].

Even than other study found that leukocytospermia has no negative influence on fertilization or pregnancy rates following either IVF or ICSI (Lackner *et al*,2008). Whereas, the study of Barraud-Lange *et al*, (2011) observed that, leukocytospermia appears to be within the physiologic range is associated with an improved capacity for sperm fertilization and pregnancy outcomes. At higher concentrations, however, leukocytospermia alters either sperm fertilization ability or the probability of clinical pregnancy, compared with non-leukocytic patients. Moreover, this study is not compatible with the conclusion of other study which found no negative correlation between semen leukocytes and pregnancy outcome .The previous study noted a positive correlation between the presence of seminal leukocytes and the percentage of ejaculated spermatozoa with ideal morphology [50].

In this study the results of Kruger strict criteria for the length and width of the sperm head four all groups were significant after *in vitro* activation, this finding may resulted from inability of dead and abnormal sperm morphology to swim up and migrate into upper layer of culture media leading to elevation the percentage of active sperm concentration and MNS.

The results of this study were agree with Al-Dujaily and Adil (2009) who found a highly significant increase in the MNS after *in vitro* activation by Kruger strict criteria using Testsimplet (Ts) stain .

Also other studies were agree with this study ,who found that *in vitro* activation of human sperm caused a significant improvement in the MNS when compared to before activation techniques (Al-Dujaily *et al*, 2013).

It has also been shown in a number of studies that semen samples with a low number of morphologically normal spermatozoa produce lower fertilization rates when used for conventional *in-vitro* fertilization[52].

However, other studies found that poor sperm morphology resulted in poor embryo quality in their systems Cohen *et al*. (1991) and Parinaud *et al.* (1993), and there is speculation as to whether IVF with sperm samples of very poor morphology, apart from resulting in a poor fertilization rate, also produces a higher rate of spontaneous abortion [55].

In current study, the results of pregnancy rate (PR) after intra-uterine insemination in normozoospermia (control) and patients with leukocytospermia , teratozoospermia and leukocytospermia - teratozoospermia, revealed a highly significant differences. The percentage of PR were 20% in normozoospermia,13.3%in leukocytospermia,6.6%in teratozoospermia and 6.6% leukocytospermia-teratozoospermia respectively .This results

may be due to sperm preparation for ART is maximize the chances of fertilization to provide as many fertilized oocytes. This goal is achieved by sperm preparation technique for the fresh collected sample from the male partner, the development of *in vitro* sperm washing methods made it is possible to deliver safely adequate numbers of spermatozoa directly to the uterine cavity. Sperm isolation techniques combining the removal of seminal plasma with the selection of highly motile sperm subpopulation were applied to artificial insemination, leading to substantial improvement in success rates.

The percentage of PR after IUI of normozoospermic men was compatible with Yaseen (2014) and Adil (2009) studies which were reported the same percentage of PR (20%).Whereas,the percentage of PR after IUI of teratozoospermic men was agreement with Kamath *et al* (2010) who was reported the PR 6.25% in patients with sperm morphology with <30% normal forms according to WHO criteria in 1999 . Other study (Lee ,*et al*.2002) reported the pregnancy rate per IUI cycle was 29.9% (49/164) in patients with sperm morphology with >9% normal forms, according to Kruger's criteria .It has been found that the PR per IUI was 10.3% in couples with MNS < 20% according to WHO ,1999(Motazedian *et al*. 2010) .

The percentage of PR after IUI of leukocytospermic men in this study was compatible with Gambera *et al.(2007*) study who reported the percentage of PR was 11.3% (7/62) by using Endtz test method.

Conclusions

The pregnant rate after intra uterine insemination in normozoospermic men was 20% and in total study was 12.8% (9 out of 70), *in vitro* sperm activation can provide an active sperm concentration with high MNS percentage, as well as reduce the percentage of round cells, endtz test can be used to detect the leukocytospermia and distinguish it from the round cells in infertile and fertile patients, using KSC by Ts stain increases the percentage of accuracy in measuring MNS ,the patient with leukocytospermia can obtain the success rate of pregnancy more than teratozoospermic patient.

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