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The role of oyster shell extract as a stimulant of sperm motility for patients with secondary infertility (asthenospermia) and diabetes (type II) before and after *in vitro* preparation for IUI

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Abstract
Diabetes mellitus diabetes mellitus the most common diseases that present in many infertile patients. This study was conducted to overwhelm the negative response for the diabetes semen by in vitro activation techniques using a medium containing motility stimulants (i.e oyster). The medium used for in vitro activation was Hams F12 with 0.5% (oyster) and two techniques were performed namely; wash and spin technique and density gradient technique. The results showed that the mean of sperm motility (grade A and grade B) after activation by using density gradient technique with medium containing 0.5% (oyster) was significantly ($P<0.001$) higher than that of before activation and after activation by using wash and spin technique. According to the data of the present study, the best results of sperm motility were noticed when using a medium containing 0.5% (oyster) with density gradient technique for diabetic men. This data can be utilized to enhance the output of diabetic type 2 patient through assisted reproductive technologies programs .
Keywords: Diabetes mellitus, Density gradient washing technique, Oyster.

Introduction

Fertility is defined as the capacity to reproduce or the state of being fertile [(1)]. Whereas, infertility is the inability to achieve pregnancy after one year of unprotected intercourse [(2)].In approximately 40% of infertile the male partner is either the sole or a contributing cause of infertility. One of male factor infertility is asthenozoospermia which is defined as a reduction in sperm motility of less than 50% [(3)]. Numerous factors can affect sperm motility such as structural defect of sperm, increased semen viscosity, different infections, immunological factors, diabetes mellitus (type 2) and others [(4)].

Diabetes mellitus is a disease characterized by elevated blood glucose levels than normal values. It is the result of defective insulin secretion and /or action. The resulting of chronic hyperglycemia is associated with damage to and subsequent dysfunction of various organs, especially the blood vessels; nerves, fertility, heart, eyes, and kidneys [(5)] .There are two major types of diabetes mellitus. Type 1 is insulin dependent diabetes. Type 2 is noninsulin dependent diabetes [(6)]. Now a day's assisted reproductive technologies (ART) have become more accessible to the general population, enabling infertile couples to hope that they can materialize their aspirations for healthy offspring later in life. It is well established that and good reproductive outcome has been female's age [(7)].

Fortunately, there is great interest in seafood all over the world now and because male infertility is such an important problem, it is believed that it is necessary to investigate the effect of one of these fruits, namely; *Oysters* stimulate human sperm motility. It has been reported that oyster shell extracts are a source of essential fatty acids, especially omega-3 and omega-6, which help significantly reduce levels of bad cholesterol and raise levels of good cholesterol, which contributes to preventing atherosclerosis[(8)]. The high potassium content of *Oysters* has made it an effective aid in controlling and controlling blood pressure, which makes *Oysters* useful for people who suffer from pressure problems or cardiovascular problems [(9).] The high levels of protein in oysters also have an enhanced role in One of the benefits of oysters is that it supports the bones, as the minerals contained in *Oysters* contribute a lot to strengthening the bones, as it contains calcium,

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phosphorous, potassium, zinc, iron, and selenium[10]. *Oysters* contain vitamin E. (Vitamin E), and selenium in good quantities, which are one of the best antioxidants in the face of inflammation and free radicals. *Oysters*' antioxidant content contributes to strengthening and strengthening immunity in the face of various pathogens. Spicy possesses anti-inflammatory properties, which makes it effective in preventing cancer and age-related diseases. One of the benefits of oysters is that it increases sexual desire, due to its high content of zinc, an aphrodisiac enhancer. It also increases testosterone and strengthens erection. An addition has been used [11]. *Oysters in vitro* to culture medium used for gamete preparation and embryonic development. However, the semen of diabetic men was facing a fluctuation failure in preparation *in vitro* for assisted reproduction purposes. Therefore, the objective of the present study is to overwhelm the negative response of diabetic semen to *in vitro* activation techniques. The study will investigate the comparison of sperm function parameters of diabetic men semen between two sperm preparation techniques namely; Density gradient centrifugation technique and swim up technique (wash and spin) using Hams F12 medium with or without addition of 0.5% *Oyster* shell extract [12].

Patients, Materials and methods

-Patients

One hundred and eighty patients were involved in this study through the period from December, 2021 till November, 2022. Their ages ranged between 18- 60 years old. The patients were divided into two main groups .The study was enrolled in Al-Kadhimiaya Hospital, Umm Al-banin IVF center ,Baghdad -Iraq. Forty men were fertile that were both diabetic (type2), and non diabetic (control) .The other one hundred and forty patients were infertile suffering from diabetes mellitus (type 2) for more than 10 years.

-Preparation of *Oyster* Shell Extract.

The main method for preparing oyster shell extract is the hydroponic method. 1000 grams of oyster shells in granular powder, moistened with boiling water and peeled until oyster shells decompose (dissolving). Then a solution of ammonia is added to the filtrate, filtered and evaporated until the mass is prepared. Oysters were stored in a well-closed container protected from light and moisture [13].

-Active ingredients of *Oyster* extract

Oyster extract is one of the very important nutrients, as it contains about 200 biologically active animal chemicals, such as phosphorous, zinc, low-calorie protein, calcium, carbonate, potassium, in addition to vitamins such as vitamin B1, E,D3 and B12, minerals and other important components so oyster shell extract is considered one of the very important nutrients. As a bacterial growth inhibitor, anti-inflammatory and antioxidant, and very important in strengthening immunity and bones for men, oyster shell extract improves semen quality (such as sperm count) and erection[14,15].

-Preparation of *Oyster* Extract for *in vitro* Sperm Activation.

The concentration of *Oyster* working solution was prepared by adding 10 mg from *Oyster* extract to 10 ml phosphate buffer solution (PBS ,0.1%) in plastic test tubes contained broad spectrum antibiotic (Ampicillin 0.004g) to prevent bacterial growth[10]. The medium which used for activation was prepared by adding 0.5 ml of *Oyster* suspension(50%) to 0.5 of Ham's F-12 Media. The solution was filtered using Millipore filter (0.25µM), and then pH was adjusted to reach (7.2-7.4) [16].

-Seminal Fluid Analysis.

Each sample of seminal fluid was collected after 3-5 days of sexual abstinence directly into a clean and dry container. The specimen was examined by macroscopic and microscopic examinations according to the standard form of WHO manual (1999and 2010) [17].

-Microscopic examination

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The semen sample was thoroughly mixed, and then a drop of 10 µl of liquefied semen was a placed on warm slide (37C) and covered with a standard cover slip (22×22 mm).The preparations were examined under microscope using a standard ocular with magnification of 40X objectives. Specimens were assessed for the following Certain sperm function parameters as recommended by [18,19] namely ; sperm concentration (million/ml),sperm motility(%), Morphologically normal sperm (%) ,Round cell counts (cell/HPF) and sperm agglutination(%).

-*In vitro* Sperm Activation Techniques.

Two methods of *In vitro* sperm activation have been used in this study namel;
1-Wash and spin technique (Swim up) with the use of Hams –F12 alone and Hams F12 with *Oyster* medium 0.5%.

2-Density gradient technique with the use of Hams F12 alone and Hams F12 with *Oyster* medium 0.5% [20].

1- Wash and spin technique

One mL of liquefied semen gently mixed with 1mL of Hams F12 medium alone or Hams F12 with *Oyster* medium then the put the sample in incubation at 37C˚ for 10 minutes. And then put the centrifugation for 7 minutes per 1600 cycle. The supernatant was discarded and 1mL of Hams F12 medium was added to the pellet carefully and put in the incubator again and left for 30 minutes period.
A drop of 10µL was taken and examined under the microscope at 400X objective to assess the sperm parameters [21].

2- Density gradient centrifugation technique

This technique is done by adding 2 mL of 80% Pure sperm solution (Nidacon, Sweden Comp.) in a test tube followed by 2 mL of 40%Pure sperm solution (Nidacon ,Sweden Comp.) as a second layer and then 1mL of liquefied human semen alone or with (Hams + *Oyster*) was added in the upper layer, this test tube was centrifuge at 4000 rpm for 20 minutes. Then the supernatant was discarded. Loading the test tube with 0.5 ml of Hams F12 medium .A drop of 10 µL was aspirated from the upper layer and put on a slide with cover slip and examined under the microscope at 400X objective to assess the sperm parameters [22].

-Statistical Analysis

Data of the present study were expressed as mean ±SEM and analyzed using Analysis of variance (ANOVA) .When F value reaching the significant level, least significant test (LSD) was used to compare between the results. The values of $P \leq 0.05$ were considered statistically significant [23].

Results

1- Mean of sperm concentrations following *in vitro* activation by two techniques and media used for the semen of fertile and infertile diabetic (type 2) and fertile non diabetic men.
The mean values of sperm concentration before activation of non diabetic fertile men was significantly ($P<0.001$) higher than that of diabetic (type 2) fertile and infertile men in the four groups. After activation the sperm concentrations were significantly ($P<0.001$) higher by using density gradient technique compared with using to the wash and spin technique in all the groups. The mean values of sperm concentrations in oligoasthenospermic men before and after activation by using wash and spin techniques with and without 0.5% *Oyster* were significantly ($P<0.001$) decreased when compared to the results before and after activation in all studied. However, the sperm concentrations in oligoasthenospermic men following the activation by density gradient technique were significantly ($P<0.001$) improve compared to studied wash and spin technique as shown in table (1).

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Table 1: Mean of sperm concentrations following in vitro activation by two techniques and medium used for the semen of fertile and infertile diabetic (type 2)and fertile non diabetic men.

Method of activation	Sperm Concentration(m/ml)				
	Fertile non D.M	**Fertile DM Type 2**	**Infertile asthenospermia**	**Infertile oligo asthenospermia**	**Infertile terato asthenospermia**
Before activation	**78±0.56**	**72.7±0.63**	**67.2±0.56**	**14.9±0.63**	**65.6±0.63**
Wash and spin technique alone	**36±0.55**	**27.8±0.63**	**26.8±0.56**	**10.8±0.63**	**24.6±0.63**
Wash and spin technique with 0.5% *Oyster*	**37.8±0.58**	**29.4±0.63**	**28.3±0.56**	**11.4±0.63**	**26.1±0.63**
Density Gradient technique alone	**50.9±0.56**	**43.4±0.66**	**43.5±0.56**	**44.2±0.63**	**37.3±0.63**
Density Gradient technique with 0.5% *Oyster*	**53±0.59**	**44.9±0.63**	**44.2±0.56**	**46.9±0.63**	**42.8±0.63**
LSD=1.4					

2- Mean of sperm motility grade (A) following *in vitro* activation by two techniques and media used for the semen of fertile and infertile diabetic (Type 2) and fertile non diabetic men.

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Table (2), revealed that the active sperm motility (Grade A) pre-activation in fertile men (45±0.56) was significantly (P<0.001) higher than that of infertile diabetic(type 2) men in four groups. The percentage of sperm motility (Grade A) increases after activation in all semen samples of the four groups. There was a significant (P<0.001) elevation by using density gradient technique of fertile non-diabetic (diabetic type 2) samples compared with the other samples of infertile men. At the same time the percentage of sperm motility grade A in diabetic (type 2) men complaining from asthenoteratospermia and asthenospermia was significantly(P<0.001) increase compared to the diabetic type 2 men complaining from asthenospermia alone and men with oligoasthenospermia by using wash and spin technique or density gradient technique with both media.

3- Mean of sperm motility grade (B) following *in vitro* activation by two techniques and media used for the semen of fertile and infertile diabetic (type2) and fertile non diabetic men.
The activity of sperm motility Grade B before activation in diabetic (type2) and infertile men (4groups) was significantly (P<0.001) lower than that of fertile with and without diabetic (type2) men. Following in vitro

Table 2: Mean of sperm motility grade (A) following in vitro activation by two techniques and media used for the semen of fertile and infertile diabetic type 2 and fertile non diabetic men.

Methods of activation	Sperm motility Grade (A)%				
	Fertile non DM	Fertile DM Type 2	Infertile asthenospermia	Infertile oligo asthenospermia	Infertile Terato asthenospermia
Before activation	45±0.56	42.7±0.66	6±0.56	3.9±0.61	8±0.60
Wash and spin technique alone	52±0.56	48.4±2.3	10±0.56	8.1±0.63	15.7±0.63
Wash and spin technique with 0.5% *Oyster*	58±0.56	49.6±0.63	13±0.56	9.9±0.63	19.7±0.63
Density Gradient technique alone	63.3±0.65	53.8±0.63	17.2±0.56	11.6±0.63	24.2±0.63
Density Gradient technique with 0.5% *Oyster*	68±0.56	60±0.63	21.4±0.56	14.7±0.63	29.6±0.63
LSD=1.7					

activation by density gradient technique with and without 0.5% *Oyster*, there was significant (P<0.001)

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increase in active sperm motility grade B in infertile men complaining from asthenoteratospermia and asthenospermic compared to infertile men complaining from asthenospermia only & oligoasthenospermia. Although, non significant increase in active sperm motility grade B was occurred in fertile men not suffering from (diabetic type 2) when compared to the fertile men suffering from diabetic (type2) following the activation by both techniques, and there was significant (P<0.001) elevation compared to other infertile groups as shown in table .(3). .

Table 3: Mean of sperm motility grade (B) following in vitro activation by two techniques and media used for the semen of fertile and infertile diabetic (type2) and fertile non diabetic men.

Methods of activation	Sperm motility Grade (B)%				
	Fertile non DM	Fertile DM Type 2	Infertile asthenospermia	Infertile oligo asthenospermia	Infertiile Terato asthenospermia
Before activation	34±0.54	30.1±0.63	10±0.56	8.5±0.63	8.8±0.63
Wash and spin technique alone	35±0.56	31.7±0.63	14.2±0.56	13.2±0.63	14.9±0.69
Wash and spin technique with 0.5% *Oyster*	35.9±0.54	32.6±0.63	17.9±0.53	16.3±0.63	18.1±0.63
Density Gradient technique alone	36.1±0.54	32.9±0.63	20.7±0.56	18.7±0.63	23.6±0.63
Density Gradient technique with 0.5% *Oyster*	36.8±0.53	33.7±0.62	23.6±0.59	21.8±0.63	25.6±0.63
LSD=1.4					

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4- Mean of morphologically normal sperm following *in vitro* activation by two techniques and media used for the semen of fertile and infertile diabetic (type2) and fertile non diabetic men.
The mean of morphologically normal sperm before activation appears significantly (P<0.001) lower in diabetic (type2) asthenoteratospermic men compared with the other groups. Although ,the density gradient technique with and without adding 0.5% *Oyster* revealed a significant improvement compared to the results before activation of its corresponding group and there was a significant (P<0.001)reduction compared to the other groups. The same observation was significantly (P<0.001) revealed regarding men complaining from asthenospermia compared with the other groups as shown in table (4).

Table 4: Mean of morphologically normal sperm following in vitro activation by two technique and media used for the semen of fertile and infertile diabetic (type 2) and fertile non diabetic men.

Methods of activation	Morphologically normal sperm %				
	Fertile non DM	Fertile DM Type 2	Infertile asthenospermia	Infertile oligo asthenospermia	Infertile Terato asthenospermia
Before activation	**70.1±0.63**	**62.5±7.0**	**68.5±0.56**	**60.4±0.63**	**3.5±0.59**
Wash and spin technique alone	**70.7±0.63**	**63.2±7.1**	**68.9±0.56**	**61.5±0.63**	**5.9±0.83**
Wash and spin technique with 0.5% *Oyster*	**73.5±0.63**	**63.9±7.3**	**71±0.56**	**66.7±0.57**	**8.0±1.0**
Density Gradient technique alone	**82.1±0.63**	**75.5±8.6**	**78.1±0.57**	**73.4±0.63**	**27±3.1**
Density Gradient technique with 0.5% *Oyster*	**83.6±0.63**	**76.1±8.7**	**82.6±0.56**	**77±0.63**	**30±3.4**
LSD=5.5					

Discussion
The results of this study revealed a significant improvement in certain sperm parameters following *in vitro* activation by the two techniques and media in in all patients groups. at the first this study shown reduction in sperm concentration in both techniques and media following in vitro activation. This is due to the failure of the dead and poor motility sperms to swim up and travel from pellet to the upper layer of culture medium. These results clarified the beneficial effect of preparation techniques by the removal of dead, immotile spermatozoa and semen debris in such way only high quality motile spermatozoa were harvested and poor quality spermatozoa were absent in the post activation medium. These results were in agreement with other studies [(24)].As a reference to removing dead and unstable sperm debris and semen debris in this way only high-quality moving sperms were harvested and the absence of poor quality sperms in the activation medium.

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Moreover this study revealed a significantly increased in sperm motility (gred A, Grade B) that founded in the diabetic (type2) men with different male infertility factors especially with asthenozoospermia. were overwhelmed by *in vitro* activation techniques and the best results were obtained following the addition of 0.5% *Oyster* with Hams F-12 medium and density gradient technique was performed. Thus, the culture medium enhances different sperm function parameters following *in vitro* activation technique namely; sperm concentration, total sperm motility percentage and grade activity of forward progressive movement. Moreover, culturing of sperms with *Oyster* extract – Hams-F12 medium result in a significant increase in the percentages of sperm motility and grade activity of forward movement (grade A and grade B) of all the groups of asthenozoospermic samples. These results were similar to the results reported by other studies [25, 26]. Which states that when using laboratory stimulation techniques, especially when adding 0.5% *Oyster* with Hams F-12 medium to the technique density gradient, the culture medium works to enhance sperm parameters, including percentages for positive movement, ratios of natural shapes, and sperm concentrations, especially in patients who suffer from a low number [27].

The *Oyster* has estrogenic activity, estrogens are known to improve sperm characteristics including sperm motility and grade activity in addition to induction of hyperactive motility. Furthermore, it has been noticed that the *Oyster* contains Ca+2, potassium, glucose, fructose, vitamin E, vitamin C and many other substances e.g.: Zn+2, sucrose, amino acid. All these substances can stimulate sperm motility and the grade activity of forward movement. The sugars are considered to be a source of energy for sperm motility. Fructose is one of the principle energy substrate for spermatozoa and an activator factor of mammal spermatozoa [28.29]. These explanations are very similar to other studies that indicate that the main active ingredient in) has estrogenic activity *Oyster shell*, as estrogen improves sperm parameters, especially movement, in addition to that *Oyster shell* contains vitamins, salts, sugars, amino acids and antioxidants, all of these components have An effective role in the ability to increase sperm motility by providing energy[30].

The current study recorded enhancement in the percentage of morphologically normal sperms (MNS) with the significant decrease in the number of round cells in diabetic (type2) in infertile men. the activation of semen of diabetic (type 2) men in different male infertility factors resulted in a significant improvement in the percentage of MNS by using density gradient technique with and without *Oyster*[31].Whereas the activation by wash and spin technique with and without using *Oyster* in the medium did not increase the percentage of MNS in different male factor infertility semen. It has been reported that high levels of ROS were leading to cause male infertility , Thus the increase in round cells (If specifically the leukocytes) can produce up to 1,000 times more ROS compared with spermatozoa under physiologic conditions[32]. The sperm membrane remodeling process during spermatogenesis may be the common origin for both abnormal spermatozoa and ROS. Failures in the process, such as head-tail attachment abnormalities, incomplete acrosome development, or sperm cytoskeleton alterations, can lead to the creation of ROS and abnormal sperm morphology [33]. The results of this study were similar to other studies stating that the use of the technique density gradient with *Oyster* for patients with diabetes in particular asthenospermia ,oligoasthenospermia , asthenoteratospermia gives better results in terms of improving parameters for semen compared to the technique wash and spin [34]. As well as studies show the patients with testicular varicose that the technique density gradient gives better results.

The data of present work appears a significant differences in certain sperm parameters before and after *in vitro* activation when compared between non diabetic fertile and diabetic (type2) infertile men who complaining from this disease for >5 years .

References

1- American Society for Reproductive Medicine (ASRM).(2011):Infertility an overview. A Guide for Patients. (suppl); S(1):60-162.

2- Uenter, M;Rassede, F.G ;Jassdw, T.R.(2012): Diagnosis and classification of diabetes mellitus Type2 ; (29):43-48.

3- American Society for Reproductive Medicine (ASRM) .(2012):A Practice Committee Educational Bulletin. Effectiveness and Treatment for Unexplained Infertility. (Suppl);S(2):121-124

4- Gunby, B; Libras, S; Terry, K; et al.(2010): Assisted reproductive technologies (ART); (95): 35- 39

5- Gurunath, S; Pandian, Z; Anderson , R; et al. (2010):Defining infertility-a systematic review of prevalence studies; (5): 575-576.

ISSN NO: 2770-0003
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6- Alberti, K.G; Zimmet, P.Z.(2013): Definition, diagnosis and Classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med; (7):539–553
7- Mahadevan, M.M;Miller, M.M; Moutos, D.M.(2013): Absence of glucose decreases human fertilization and sperm movement Characteristic in vitro.I-Turn; (12): 119-123
8- Virtanen, S.M; Knip, M.(2012): Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia; (78):1053-1060.
9- Gunby, B; Libras, S; Terry, K; et al.(2010): Assisted reproductive technologies (ART); (95): 35- 39
10- AL-Dujaily, S.S; AL-Janabi, A.S; and Nori, M.(2012): Effect of *Oyster* extract on in vitro sperm activation of asthenospermic patients; (3): 477-483.
11- Al-Dujaily,S.S; Mizheir ,Z. H.(2012): Effect of *Oyster* Extract on in vitro Sperm Activation and Embryonic Development Following Inntra-peritoneal Insemination in Mice; (4):34-36
12- Anagha, K; Manasi ,D; Priya, L.(2013): Review About pharmacological study of *Oyster* shell Extract virous animal model; (3): 152- 164.
13- Chin, Y.W; Jung, H.A; Liu, Y; et al. (2011):Anti-oxidant constituents of the roots and solons of (Oyster); (12): 4691-4697.
14- Rajesh, M.G; Latha ,M.S.(2023): Protective activity of *Oyster* Linn. On carbon tetrachloride induced per oxidative damage; (5): 284-287.
15- Jatav, V.S; Singh ,S.K,; Khatri ,P; et al. (2012): Recent pharmacological trends of *Oyster;* (1): 170-185.
16- Cui ,Y.M; Aoen, M.Z.(2012): Effect of glabridin from *Oyster* on learning and memory in mice; (4): 377-380.
17- Ravichandra, V; Ahalya, D; Adiga, S; et al.(2013): Evaluation of the effect of Oyster Linn shell extract on spatial learning and passive avoidance response in rats; (44): 214-219.
18- Menkveld, R; El- Garem, Y; Schill, W;et al.(2013): Relation ship between acrosomal function and sperm morphology; (20):432-438.
19- WHO laboratory manual for the examination and processing of human semen 5th ed. (2010):
20- Comhaire, F; Vermeulen, L.(2011): Human semen analysis; (1): 343-362.
21- Cooper, T.G; Noonan, E; Eckardstein, S; et al.(2011): World Health Organization reference values for human semen characteristics; (3): 231–239.
22- Al-Ani, K; Neeka, G.(2015): An in vitro human sperm activation and intra urtrine insemination study using Global and Hams F-12 media. High Diploma thesis Institute of Embryo Research and Infertility Treatment, Baghdad University.2007; 12: 344-351.
23- Barbara I llowsky; Susan Dean (2014). Introductory Statistics.
24- Hargreaves, T; Mahmoud, A.(2012): Manual for the Standardized Investigation, Diagnosis and Management of the Infertile Male;(5):228-231.
25- Coulam, C.B; Moore, S.B;O'Fallon, W.(2014): Investigating Unexplained Infertility; (1):374-381.
26- World Health Organization.(2010): Towards more objectivity in diagnosis and management of male infertility of a world health organization multicenter study. Int. J. Androl. Suppl.; 7(10): 1-3.
27- Agarwal, A; Sushil, A;Parbakaran, C.(2015): Clinical Relevance of Oxidative Stress in Patients with Male Factor Infertility; (8): 34-55.
28- Cavallini, G.(2012): Male idiopathic Oligoasthenoteratozoospermia; (2):143-157 .
29- Greenspan, F.S; Gardner, D.G.(2016): Pancreatic hormones and diabetes mellitus type2 : basic and clinical endocrinology; (5): 692-698.
30- Alberti, K.G; Zimmet, P.Z.(2013): Definition, diagnosis and Classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med; (7):539–553.
31- Mahadevan, M.M;Miller, M.M; Moutos, D.M.(2013): Absence of glucose decreases human fertilization and sperm movement Characteristic in vitro.I-Turn; (12): 119-123.
32- Yeh, G.Y; Eisenberg , D.M; Kaptchuk, T.J; et al. (2013):Systematic review of herbs and dietary supplements for glycemic control in diabetes; (26):127-129.
33- American Society for Reproductive Medicine (2015). Report on DM (typ2)and infertility. Fertility and Sterility, 90(Suppl 3): S247–S249.

ISSN NO: 2770-0003
May 2024

34- Goldstein, M. and Eid,J.F.(2011). Elevation of intratesticular and scrotal skin surface temperature in men with varicocele, Journal of Urology, vol. 142, no. 3, pp. 743–745,