وزارة التعليم العالي والبحث العلمي
جامعة ميسان
كلية العلوم
قسم علوم الحياة

تاثير العمر ومدة الاصابة بمرض السكري من النوع الثاني على بعض المعايير الهورمونية و الكيموحيويه والخصوبة لدى الرجال

رسالة مقدمة
الى مجلس كلية العلوم / جامعة ميسان
وهي جزء من متطلبات نيل درجة الماجستير علوم في علوم الحياة

من قبل
حسين حميدي جليب الكعبي
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بسم الله الرحمن الرحيم

﴿إِنَّا خَلَقْنَا الْإِنسَانَ مِن نُّطْفَةٍ أَمْشَاجٍ نَّبْتَلِيهِ فَجَعَلْنَاهُ سَمِيعًا بَصِيرًا﴾

صدق الله العلي العظيم

(سورة الانسان: اية ٢)

Chapter One

Introduction

1-1: Introduction

Diabetes mellitus (DM) is a major challenge for global public health, and it comprises a heterogeneous etiology of diseases characterized by elevated blood glucose **(**Dragana *et al.*,2015**).** Type I diabetes is caused by autoimmune destruction of insulin- producing pancreatic β cells, insulinopenia with resultant hyperglycemia. It is diagnose during infancy and requiring exogenous insulin for survival (Zipitis and Akobeng, 2008**).**

Type II diabetes is a complex disorder characterized by imbalance between insulin resistance and insulin secretion that induce liver glucose output by preventing glycogen formation and stimulating glycogenolysis and gluconeogenesis **(**Holt and Hanley, 2012).

Type II diabetes mellitus have increased prevalence of dyslipidemia. (Maharjan *et al*.,2017). DM changes in lipid levels and consequent disorders o f lipid metabolism and stress have been observed (Betteridge., 1994). Such as increases in circulating levels of free fatty acids (FFA), triglycerides and dense low-density lipoprotein cholesterol particles together with reduced levels o f high-density lipoprotein cholesterol levels (Nikolic *et al* ., 2013). Many authors suggest that DM decreases serum testosterone levels. This is associated with a steroidogenetic defect in Leydig cells. (La Vignera *et al.,* 2012).

DM may affect male reproductive function at multiple levels as a result of its effects on the endocrine control of spermatogenesis, or by impairing penile erection and ejaculation (Bener *et al.*,2009).

Type II diabetic could be a progressive disease during which the risks of stroke, small vascular events, and mortality are all powerfully related to hyperglycaemia (Stratton *et al*., 2000).

The disease course is primarily characterized by a decline in β-cell perform and worsening of hormone resistance, also diabetes is cause several general complication, male physiological condition supported impotence, retrograde ejaculation, and incompetence. DM induce refined molecular change that are necessary for spermatozoon quality and performance (Mallidis *et al.*, 2011).

DM causes effect of the epididymis, with a negative impact on spermatozoon transit. Varied mechanism is made as case for the spermatozoon harm discovered in diabetic patients, these comprise endocrine disorder, neuropathy and increased oxidative stress. Also type II associated with hypothalamic-pituitary-gonadal (HPG) axis suppression , where decrease in testosterone and other sex hormone (AL-Aaraji,2016) .

1-2: Aims of the study

The aim of this study is to known the effect of diabetic mellitus type 2 on some hormonal, biochemical and seminal plasma in serum and semen as the following:

1. In blood to measure (HBA1c)
2. In serum
    a. Fasting blood glucose (F.B.G)
    b. Lipid profile (cholesterol, triglyceride, HDL, LDL and VLDL)
    c. Hormones (FSH, LH, PRL, Testosterone and E2)
3. Fertility:
    a. Macroscopic examination of the semen (pH, volume, liquefaction and viscosity)

b. Microscopic examination of the semen (concentration, motility and morphology)
c. Biochemical parameters of the seminal plasma (fructose, zinc, alkaline phosphatase and glutathione)

Dediction

To……

To ……whom Allah sent as mercy to the

Worlds ……Prophet Mohammed

To the candle that melted to lighten my road

……My father

To the affectionate heart that filled me with love…

My mother.

To the roses that perfumed my life ……My brothers

To the one who supported me……My friends

With my love……Hussein

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Thanks also dedicated to all of the patients and healthy for their cooperation.

I would like to thank my colleagues in the study who stood with me to complete this thesis, especially Hussein Ali AL-quzwini.

Finally, I would like to thank all persons whom they could assist me in any way and I cannot remember them at this moment of writing.

Hussein

Supervisor 's Certificate

We certify that this thesis entitled " **The Effects of Duration and Age of Diabetes Mellitus Type 2 on Some Hormonal, Biochemical and Fertility Parameters in Men**

"has been prepared under our supervision at the College of Science, University of Misan; as a partial fulfillment of the requirements for the degree of Master of Biology

.

Signature

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Date: / 2 /2019

Recommendation of Head of Biology Department

I view of the available recommendations; I forward this thesis debate by the examining committee.

Signature

Assist. prof. Dr.Zahid S. Aziz

Head of Department of Biology

College of Science/Misan University

Date: / 2 /2019

Chapter Five

Discussion

5-1: Biochemical parameters in serum (According to Age)

5-1-1: Fasting blood glucose and HbA1C levels

The result of the present study showed that the diabetic patients had significantly high concentration of FBG and HbA1C ($P<0.05$) than control group. The DM is a group of metabolic disorders characterized by hyperglycemia, the elevation in FBG level may be resulting from defection in insulin secretion, insulin action or both (ADA, 2014). Blood glucose is controlled by two processes: first insulin secretion by pancreatic B cell in response to a nutrient challenge and second by insulin action on target organ i.e liver and adipose tissue (ADA.2014).

T2DM is often associated with result from insufficient insulin production and insulin resistance (Vital *et al*., 2006).

According to the American Diabetes Association (ADA) guidelines 2007, the value of HbA1C should be kept below 7% in all diabetes (ADA , 2007). However, the patients under study are poorly controlled because HbA1C of the patients groups > 9.9 % and this result is previously reported in other reports and correlated with different complication of DM in patients with elevated levels of HbA1C in T2DM (Saaddine *et al.*, 2002). In comparison with a study, Maki *et al*, (2009) found they HbA1C in diabetes groups was 6.7±0.1%.

5-1-2: lipid profile

In the current study, the result showed that lipid profile TC,TG,LDL,VLDL which increased significantly but HDL decrease significantly in diabetic patient as compared with control. These finding were in agreement with the findings of Dixit *et al*.(2014) and Hashim (2015).

In our study show significantly increased the lipid profile and significantly decreased in HDL when compared with control. .

In many studies have shown a relationship between dyslipidemia with diabetic mellitus which high of cholesterol, triglyceride, LDL, VLDL and decrease of HDL (Al Mukhtar, 2005; Nema, 2008; Ismail, 2014; Hashim, 2015) .

In diabetic many factors may affect blood lipid profile because of interrelationship between carbohydrate and lipid metabolism. Therefore , any disorder in carbohydrate metabolism lead to disorder in lipid metabolism and vice versa (Chatterige and Shinde,2005).

In type 2 diabetes the major disturbance in lipoprotein metabolism are reflected by increase in plasma triglyceride and a low HDL-cholesterol with normal or near normal LDL-cholesterol levels .However in diabetes this LDL fraction contain a greater proportion of small, dens LDL particle which are believed to be more atherogenic (Syvanne and Taskinen , 1997).

On other hand metabolic disturbance of lipid which increase flux of free fatty acids (FFA) back to the liver resulting enhanced secretion of very low density lipoprotein (VLDL) from liver in to blood stream by two enzymes cholesteryl esters transferase and hepatic lipase (Jenkins *et al.*,1996)

The general increased of levels of serum lipids in diabetic patients may be mainly attributed to increase in the mobilization of free fatty acids from fat tissues, Then, excess fatty acids in serum are converted into triglycerides, phospholipids, and cholesterol in liver (Verges, 2015).

Therefore, the current results concerning the lipid profile might be faced a risk factors for cardiovascular diseases in patients with diabetes II .

5-1-3: Hormones

The present study showed a significant decrease in testosterone, FSH, LH levels and a significant increase in PRL and E_2 in T2DM incomparison with control group. Hofny *et al.* (2010) observed a decrease in TT levels in men with DM II .

The presence of low free testosterone level suggests that insufficient testosterone may be a risk factor in these patients (Cai *et al*., 2014;Shaheen *et al.*, 2014).

The testosterone level was significantly decreased in men compared to controls. Also LH and FSH were significantly increased in men, these results are in agreement with the study of (Hofny *et al*., 2010 ; Esmaeel, 2013).

AL-Aaraji,(2016), noted that TT decreased and E2 be increased in diabetic patients with type II when compared with control.

Men with diabetic have decrease testosterone concentration than control, may be due to trans (TT) into estradiol (E2)by the action aromatase in adipose tissue therefore, a reduce of (TT) with increase aromatase , which is a result of high number adipocyte in diabetic men .(Kelly and Jones, 2013).

Type 2 diabetic is associated with decrease testosterone and increase estradiol high esradiol/testosterone ratio associated with abdominal and subcutaneous fat and cause more aromatization of testosterone to estradiol (AL-Aaraji, 2016).

La vignera, (2012) referred to DM decrease TT, this associated with steriogentic defect in lyding cell. Dhindsa *et al*., (2004) demonstrated that 33% of men with type 2 diabetes had significantly lower levels of free testosterone. Ota *et al*., (2002) show that low testosterone associated with insulin resistance diabetic men.

Pitteloudet *et al*.,*(*2005) observed the leydig cell population and testosterone metabolites were reduced, which was inversely related to the increase of insulin resistance.

Ding *et al*., (2006) were indicated that endogenous sex hormones may differentially modulate glycemic status and risk of type 2 diabetes in men & women. Hussein and Al-Qaisi (2012) observed a significant reduction in FSH and LH levels diabetic men when compared with control.

AL-Shammaa, *et al.(*2015) found low FSH,LH ,T may be due to Leydig and Sertoli cell which decrease with insulin deficiency which was referred to as a cause of impotence in diabetics , also defects in insulin secretion may change testicular and accessory sexual glands function. Also, Al-Fartosy and Mohammed (2017) carried out in maysan ,Iraq show reduced luteinizing hormone and follicle stimulating hormone to response to gonadotropin-releasing hormone in diabetic men indicated a decreased acute releasable pool of pituitary gonadotropins.

In other hand study by, AL-Aaraji,(2016), didn't agreement with our result, study carried on 40 patient with T2DM observed high level FSH, LH compared with control , because leydig cell and follicle cell more resistance to gonadotropin hormone .

Study by Onah *et al*,(2013) showed in Nigeria on 175 men where high level of prolactin level diabetes mellitus type 2 with compared with control , and high level of prolactin cause hypothalamic pituitary

dysfunction and can suppress gonadal function direct. The cause of the elevated mean serum PRL level and occasionally observed hyper prolactinaemia in diabetics is not clear. Differences in dietary habits would be one possible explanation since diet is known to influence serum PRL levels (Edith, 2010).

5-2: Biochemical parameters in serum (According to Duration of DM)

5-2-1: Fasting blood glucose and HbA1C levels

The current result agree with study by Verma *et al*. (2006) which observed a significantly increase in FBG and HbA1C values in male and female diabetic patients with time progressing. Study prove that the amount of carbohydrate attached to the HbA1c increases with increasing duration of the disease (Sampson, 2002).

5-2-2: Lipid profile

The duration of diabetes was associated with higher incidence of dyslipidemia (Talat *et al*., 2003).

Sultana (2010), Uttra *et al*.,(2011) , Jain *et al*., (2016) observed significantly increased in TC, TG, LDL, VLDL and significantly decreased in HDL in patients with DM with time progressing. While Otamere *et al*.(2011) found the duration of disease don't have effection the lipid profile.

Increase of total cholesterol levels to oxidative stress conditions which had been occurred in DM and positively correlated with a long period of the disease, in addition to a defect in LDL receptor as a result of glycation of the receptor.(Al-Zangana,2006)

The prevalence of hypercholesterolemia, hypertriglyceridemia, high LDL-C and low HDL-C levels among subjects with high HBA1C Insulin affects the liver apolipoprotein production. It regulates the enzymatic activity of lipoprotein lipase (LpL) and Cholesterol ester transport protein. All these factors are likely cause of dyslipidemia in Diabetes mellitus (Amoako, 2015). Moreover, insulin deficiency reduces the activity of hepatic lipase and several steps in the production of biologically active LpL may be altered in DM (Durrington and Snider-man, 2002).

5-2-3: Hormones

The current study shows that the testosterone, FSH, LH level signifi-cantly decreased and increased significantly in PRL and E2 in T2DM comparison with control group.

Dhindsa *et al*, (2004), Jihan *et al*, (2016) study was done with male patients. Found decrease of testosterone when compared to control with time progressing.

The cause of decline in T is a combination of testicular and pitui-tary/hypothalamic defects

Dhindsa *et al*, (2004) The study was done for male patients with type 2 diabetes referred to the center for management of diabetes, found de-crease of FSH and LH when compared to control, because of hy-pogonadism in these patients could be decreased gonadotropin secre-tion.

Ballester *et al*, (2004) observed a significant reduction in FSH and LH in rat induce diabetes, may be due to absence of stimulating affect of insulin on leyding cell which result decrease in FSH and LH levels.

Our results show increase a significant of prolactin compared with control study by Al Hayek *et al*,(2017) carried out on 157 men with diabetes mellitus, where found increase significantly compared with control. High level of prolactin cause of infertility in male and female mammals, elevated prolactin may impact reproduction through an action on the GnRH neurons of the hypothalamus and/or on the pituitary gland to affect secretion of the gonadotropins, LH, and FSH (McNeilly,2001)

5-3: Fertility parameters in semen (according to age)

Our result recorded that pH and liquefaction in semen of diabetes patients decreased and increased significantly. Singh *et al*.,(2014) , found no significant variation in pH of semen values between diabetic(7.8±0.4) and control(7.4±0.7).The epididymis contains the acetic acid, which spreads to the sperm to reduce the degree of pH(Sircar, 2008). And increased significantly in liquefaction in patients compared with control may be due to absence of activity of fibrolytic enzymes found in seminal fluid(Plich and Mann, 2006).

Our result recorded that, also the concentration, progressive, no progressive motility and normal morphology are significantly decreased, while increased in sluggish motility, dead and abnormal morphology.

This result agree with Garcia-diez *et al*.,(1991), Singh *et al.*,(2014), Zhu *et al*.,(2017)which found decrease significantly in sperm concentration, sperm motility, sperm morphology.

Also Bhattacharya *et al*, (2014) Found decreased significantly in percentage motility and normal morphology compared with control.

In other hand Ali *et al*., (1993) reported increased sperm concentration in men with insulin dependent diabetes mellitus (IDDM) and

non-insulin dependent mellitus (NIDDM) with neuropathy, compared with control. While Delfino *et al*, (2007) observed sperm concentration in male with diabetes mellitus did not differ significant compared to control. Suggests spermatogenesis disruption and germ cell apoptosis in DM are related to local autoimmune damage.

Insulin stimulate the ledying cell function, where defect insulin caused defect spermatogenesis (Perrard-Sapori *et al*., 1987). Sperm cell susceptible to oxidative stress and sperm cell contain high concentration of polyunsaturated fatty acid in membrane (Aitken *et al*, 1989).

DM also provokes detrimental blood testis barrier alteration, which may be responsible for spermatogenesis disruption (Alves *et al*., 2013).

Our study show the motility were significantly lower in patient DM than control. Impaired sperm motility observed in DM patients might be attributed to many reasons such as increase ROS level, altered mitochondria DNA and decrease of the epididymal products (Singh *et al*., 2009; Jangir and Jain, 2014).

Many studies (Delfino *et al*, 2007; Mangoli *et al*, 2013) showed that DM leads to marked reduction in fecundity by altering the normal morphology of sperm cell. Also, increased oxidative stress also harmful to sperm morphology and is considered a main factor of decreased of normal sperm morphology in DM.

Also increased lipid peroxidation in patients with diabetes associated with low normal sperm morphology and increased of abnormal morphology (La Vignera *et al*, 2012). Abnormal sperm would be source of superoxide anions that bind with zinc present in seminal plasma and thus decrease the zinc levels (Chia *et al*., 2000).

5-3-1: Seminal plasma

Our result showed a significant reduction in Zinc and GSH in patient's diabetes compare with control, while the fructose and ALP do not reach to the significantly level.

Our result agree with many studies reported the level of zinc in seminal plasma which decreased significantly in patients of diabetic men when compared with control (Sujatha, 2013; Ozturk *et al*., 2013; Ghasemi *et al*., 2016). Zn microelement is very essential for male fertility, and their deficiency impedes spermatogenesis and is a reason for sperm abnormalities and has a negative effect on serum testosterone concentration(Fallah *et al*.,2018).Low zinc in men may be caused prostate cancer, infertility and linked to low libido (Abdelhalim, 2010).

Our result agree with (Popoola *et al*, 2017; de Oliveira *et al*, 2016)which noted a significant reduction in GSH in diabetes rats when compared with control.

Higher levels of GSH in seminal plasma may be play a role in protection against oxidative damage and to improve the sperm motility and morphology (Atig *et al*, 2012). Hyperglycemia is known to augment OS by increasing ROS formation and altering the normal redox state Several mechanisms contribute , such as an increased polyol pathway flux, increased intracellular formation of advanced glycation end (AGEs) products, activation of protein kinase C, and an overproduction of superoxide in the mitochondria (Brownlee 2001).

Diabetes induce alteration in activity of enzyme glutathione peroxidase and reductase, these enzyme found in all cell that metabolizes peroxide to water and converting glutathione disulfide back into glutathione so,

alteration in their levels make cell prone to oxidative stress and caused cell injury (Asmat *et al*, 2016).

5-4: Fertility parameters in semen (According to duration of DM)

This study shows that the pH, concentration and motility (progressive and non-progressive) is significantly decreased. It is also the normal morphology significantly decreased. Increased in sluggish, dead motility and abnormal morphology in patients when the diabetes is compared with control group.

Study by Padron *et al.,* (1984) and Condorelli, *et al*., (2018) noted decreased significantly in sperm concentration, sperm motility and sperm morphology compared to control with time progressive of duration diseases.

Decreases of sperm concentration in diabetes mellitus compared to control may be due to low in FSH and LH which responsible for spermatogenesis (Maneesh *et al*., 2006). Sperm cell susceptible to oxidative stress, and sperm cell contain high concentration of polyunsaturated fatty acid in membrane which increased with progressive time of duration (Aitken *et al*, 1989). The decrease motility observed in DM patients might be attributed to increase ROS levels, altered mitochondrial DNA (Jangir and Jain, 2014).

Sikka, (1996) found high level of reactive oxygen species in abnormal sperm compared to normal sperm, the increased of abnormal sperm may be due to increased oxidative stress which accompanied by an increase reactive oxygen species (ROS). In other hand decrease motility may be due to be due to increased oxidative stress which which accompanied by an increase ROS Which lead to decreased mitochondrial

membrane potential (MMP) that associated with decrease motility of sperm(Condoreli, 2018; Kasai *et al*., 2002).

5-4-1: Seminal plasma

This study shows significantly differences in Zinc and GSH between patient with diabetes and control, and did not differ significantly in fructose and ALP values.

Adewole *et al*., (2007) mentioned that Zinc and GSH decreased significantly in diabetes induce rat along with time progressive of diabetes.

Diabetes induce change in antioxidant agent which playing an important role in remove reactive oxygen species (ROS), and decrease of antioxidant leading the cell exposed to defect (Asmat *et al*, 2016). (Amaral *et al.*, 2006).

Insufficient protection by antioxidants render spermatozoa vulnerable to oxidative damage (Eskiocak *et al*, 2005).Antioxidants improved insulin sensitivity, by reducing oxidative stress and insulin resistance (Udupa *et al*., 2012).

Low zinc in men may be caused prostate cancer, infertility and linked to low libido (Abdelhalim, 2010).

Zinc improves sperm quality on its own as a membrane stabilizer, and as a component of superoxide dismutase prevents sperm apoptosis and sperm DNA fragmentation (Anim,2014).

Chapter Three

Materials and methods

3-1: Apparatus and Equipment

The apparatus and equipment used in this study are summarized in table (3-1)

Table (3-1): The apparatus and equipment that used with their producing companies and countries.

NO.	Apparatus and Equipment	Company (origin)
1	Alcohol methyl	Meheco,China
2	Bio- Rad	USA
3	Blance	Camry,china
4	Centrifuge	Beckman,England
5	Container cup	Midea,china
6	Disposable syringe	Medico (United Arab Emirates)
7	EDTA tube	AFCO,Jordan
8	EILSA	Germany
9	Gel tube	Sun, Jordan
10	Glass slide and cover slips	Sail Brand, china
11	Hemocytometer	Marenfeld Burker,germany
12	Incubator	Heraeus,Germany
13	Light microscope	Olympus, Japan
14	Minividus	Biomerieux, France
15	Plain tube(10 ml)	AFMA, Jordan
16	Plastic container	Meheco,China
17	Refrigerator	Midea,China
18	Spectrophotometer	CECIL/CE-1011, England
19	spectrophotometers UV	Shimadzu, Japan
20	Timer with alarm	Hitachi ,Japan
21	Water bath	Memmert, Germany

3-2 Kits

The kits used in this study are summarized in table (3-2)

Table (3-2): The kits that used with their producing companies and countries.

No.	Kit	Company(origin)
1	ALP,	Shanghai Biological,China
2	Estradiol (E2)	Biomerieux,France
3	Follicular stimulating hormone(FSH)	Biomerieux,France
4	Fructose	LTA , Italy
5	Glucose	Spinreact ,Spain
6	GSH	Shanghai Biological,China
7	High density lipoprotein (HDL)	Bio Lab, France
8	Low density lipoprotein (LDL)	Bio Lab, France
9	Luteinizing hormone (LH)	Biomerieux,France
10	Prolactin (PRL)	Biomerieux,France
11	TotalTestosterone (TT)	Biomerieux,France
12	Total Cholesterol (TC)	Bio Lab, France
13	Triglyceride (TG)	Bio Lab, France
14	Very Low density lipoprotein (VLDL)	Bio Lab, France
15	Zinc	LTA , Italy

3-3: Population of the study

This study was carried out at the AL-sadder Teaching Hospital and center for Endocrinology and diabetes specialist in Missan province .

The population of this study consist of 60 men with average age between from 30-59. The population is distrbutaed as follows: 45 population with type II diabetic and 15 health men. The 15 healthy men represents the control group. The researcher excluded some of the patients because of the history of cardiopathy, nephropathy, hypertension, smoking, thyroid gland disease, prostate surgery and varricolla (Appendix I) .

3-4: Experimental design

The study included 60 men (45 diabetic and 15 healthy as control group), ranging in age from 30 to 59 years. The number of infected divided by age was divided into three groups, the first (30-39) years , second (40-49) years and third (50-59), also divided by the duration of diabetes to three groups also the first (1-5) year , second (6-10) year and third (11-15) years, when measuring F.B.G, HbA1C lipid levels and reproductive hormones. While included measurements of fertility, age groups, first and second, as well as the duration of diabetes the first and the second groups, as follows in Figure (3-1):

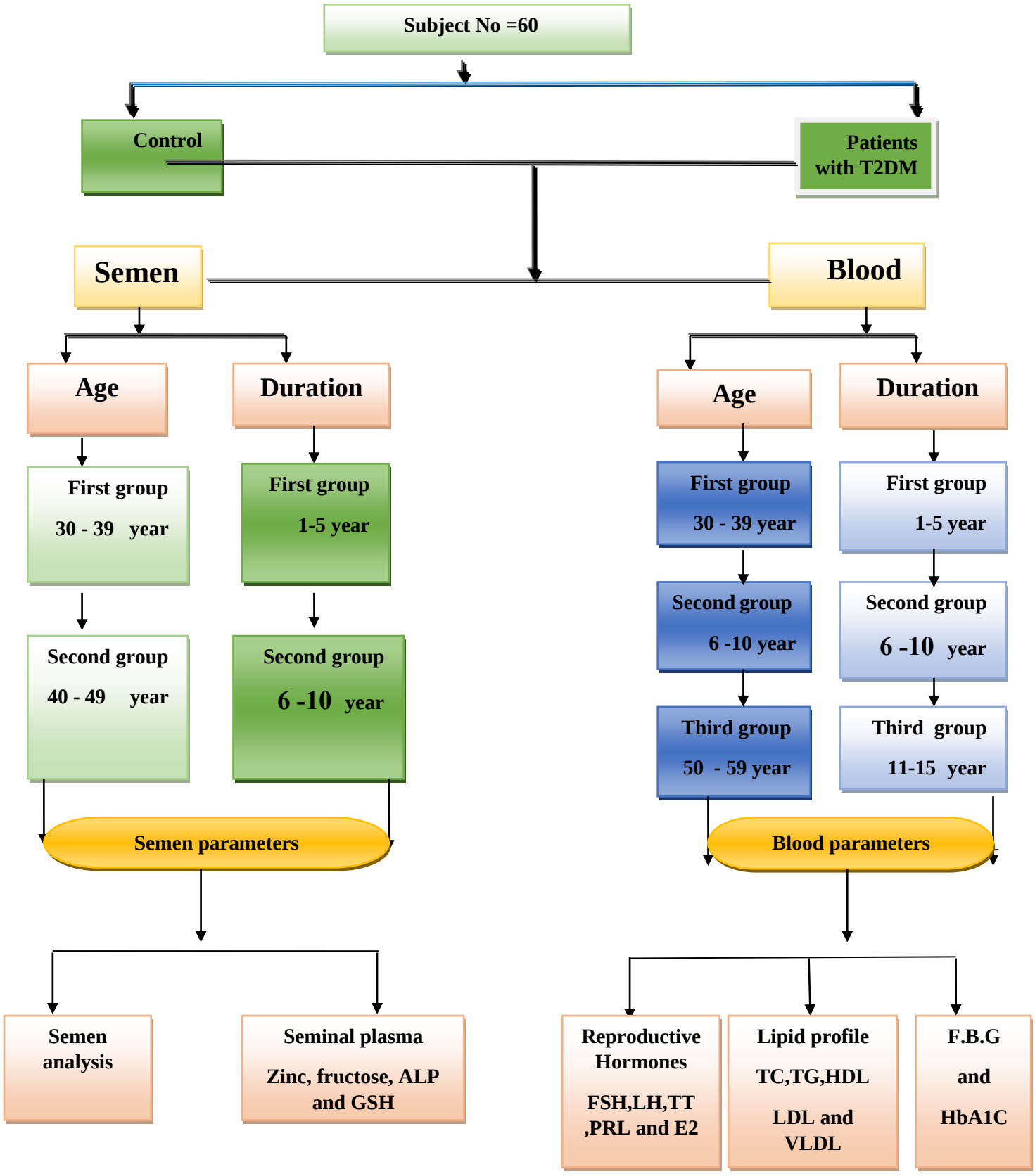


Figure (3-1): Experimental Design

3-5: Sample collection

3-5-1: Blood Collection

Seven to ten milliliter of whole blood was obtained by peripheral Venipuncture from each fasting patient and control at (8-10 AM). The blood sample was divided into fractions; 2.5 ml was transferred into EDTA tube for HbA1C determination, and other fraction of blood sample was left for 15 minutes to clot at room temperature ,and then serum was separated by centrifugation at(3000 rpm) for (5 min) , to measure glucose .last serum was transferred into labeled plain tube and stored at -20 Ċ° until used for estimation of hormones (FSH,LH,TT, PRL and E2) and lipid profile.

3-5-2: Semen collection

The ejaculates were collected after abstinence period of (3-5days). In a sterile, non-toxic, disposable Petri-dish by masturbation achieved in a private room near the laboratory prepared for this purpose to minimize the contact of the seminal fluid to temperature inconstancies and to manage the point in time between assortment and analysis, the specimen was labeled with patient's name and lab number (WHO, 2010).

3-6: Methods

3-6-1: Biochemical parameters

3-6-1-1: Serum glucose measurement

The serum glucose was determined by enzymatic colorimetric (GOD-PAP) method, using kit supplied by Spinreact, Spain (Trinder, 1969)

Principle of test

glucose was oxidized by glucose oxidase (GOD) to gluconate andH_2O_2. The formed H_2O_2 then react under catalysis of peroxidase with phenol and 4-aminophenazone to yield a red –violet quinonimine dye

Assay procedure

Solutious	Sample	Standard	Blank
Workin greagent Standard Sample	(lml) 10µL	(lml) 10µL	**(lml)**

Mixed and incubated for 10 min at 37 ºC or 20 min at room temperature (15-25 ºC) reading of the absorbance(A) of the sample and standard against the blank at 505 nm.The colour was stable for 30 min.

Calculations

Glucose mg/dl= $\boldsymbol{Asample}$ /$\boldsymbol{Astandard}$× **100** ($\boldsymbol{standardconc}$.

3-6-1-2: Determination of HbA1c

The component of HbA1C kit

NO.	**Items**	**Specification**
1	Elution Buffer 1	2 bottles(2000ml)
2	Elution Buffer 2	1 bottle (1000 ml)
3	Wash /Diluent solation	1 bottle (1600 ml)
4	Calibrator /Diluent set,Hb,A2/F/A1c	Calibrator Level 1; 3 vial (7 ml) Calibrator Level 2; 3 vial (7 ml) Calibrator Diluent ;1 bottle (100ml)
5	Sample vial	Two packs(1.5 ml)

6	Floppy diskette	Kit –specific D-10 Dual program parameter information
7	Whole blood primer	4 vial (1.0 ml)
8	Thermal paper	Box of 10 roils

Principle of procedure

The d-10 Dual program is based on chromatographic separation of the analytes by ion –exchange high –performance liquid chromatographic (HPLC)

The sample is automatically diluted on the D-10and injected into the analytical cartridge. The D-10delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobin is separated based on their ionic interaction with the cartridge material. The separated hemoglobin then passes through the flow cell of the filter photometer, where cgange in the absorbance at 415nm are measured. A calibrator is analyzed with each run for adjustment of the calculation parameters for determination of HbA1C, the value recorded is in percent. According to (Chandrashekar, 2016).

Procedure

- The HbA1C kit put out of refrigerator for 10 min
- The reagents are placed in their place on the side of the external device .
- The two milliliter (2ml)of blood was taken by medical syringe and place it in EDTA tube and then ,the sample mixed gently by inverting the tube.
- The sample tubes allowed to reach the room temperature (25 c) before performing assy.

- The sample tube is loaded into the D-10sample rack and put it in the place known inside the device D-10
- Patient QC ID was appearing on the screen after they have been acanned by the barcode reader
- The DONE button was press after you have entered each patient ID
- The START button was press to begin the analysis
- The steps for the device followed to start the calibration process automatically.

3-7: Lipid profile

3-7-1: Measurement of serum Total cholesterol (TC)

Procedure

The employed method was based upon an enzymatic colorimetric method ,using cholesterol assay Bio lab Kit. The principle of the kit is according *(*Allain *et al .,1974).*

The following reagent was pipette in the test tube

	Blank	**Standard**	**Sample**
	1ml	1ml	1ml
Standard	-	10 µL	-
Sample	-	-	10 µL

Incubated for 10 minutes at room temperature . The absorbance of the sample and the standard sample were measured against Blank reagent at (480-520).

The color intensity is proportional to the cholesterol level in the sample that can be estimated using the spectrophotometer, color of the mixture was varying from light pink to dark red , and it was stable for 1 hour.

Calculation

Conc. ofcholesterol (mg/dl)= Abs. of sample /Abs .of standard × Conc.of standard .

Note:

Cholesterol standard is 200 mg/dl (5.17 mmol/L) concentration according to the manufacture's leaflet of Biolabo Kit .

Expected values

The next guideline may be used for clinical evaluation :

Risk classification of the total cholesterol

Total Cholesterol	**mg/dl**	**Mmol/L**
Recommended rate	<200	<
Low risk	200-239	5.18-6.19
High risk	>240	>6.22

3-7-2: Measurement of serum Triglycerides (TG)

Procedure

The method was based upon an enzymatic colorimetric method ,using cholesterol assay Biolabo Kit . The principle of the kit is according(Tiezet,1999).

The following reagent was pipetted in the test tubes

	Blank	**Standard**	**Sample**
Reagent 1	1ml	1ml	1ml
Standard	-	10 µL	-
Sample	-	-	10 µL

Incubated for 10 minutes at room temperature . The absorbance of the sample and the standard sample were measured against Blank re-agent at (480-520)nm.

The absorbance of the colored complex (quinoneimine) is proportional to the concentration of triglycerides in the sample can estimated by spectrophotometer,color of the mixture was varying from light pink to dark red, and it was stable for about 1 hour.

Calculation

Conc.of triglycerides (mg/dl) =Abs.of sample /Abs.of standard ×conc.of standard

Note

Triglycerides standard is 200 mg/dl(2.28 mmol/L) according to the manufacture's leaflet of Bio Lab Kit .

3-7-3: Measurement of serum HDL-cholesterole

Procedure

The method was based upon an enzymatic colorimetric method ,using HDL-cholesterol assay Kit Bio labo. The principle of the kit is according (Lopes-Virella *et al*.,1977).

The following reagent was pipetted in the test tube

	Blank	**Standard**	**Sample**
Reagent 1	1ml	1ml	1ml
Standard	-	25 µL	-
Sample	-	-	25 µL

Incubated for 10 min at room temperature The absorbance of the sample and the standard sample were measured against Blank reagent at (480-520)nm.

Followed by 3500 rpm for 15 min .The clear supernatant was used for cholesterol was used for cholesterol determination using cholesterol assay kit as described in the and the absorbance of the sample is measured by spectrophotometers at 505 nm .

Calculation

HDL-C concentration was estimated according to the following equation:

Conc. of HDL-C (mg/dL) =Abs. of sample /Abs. of standard × conc.of standard

Note

HDL-C standard is 100mg/dL (2.58mmol/L)) according to the manufacture's leaflet of biolabo Kit .

Expected values

Male	**41.0-58.7 mg/dL**	**1.06-1.52**

3-7-4: Measurement of serum Low Density Lipoprotein- Cholesterol (LDL-C)

Concentration of LDL-C was determined according to the Friewald equation (Friewald *et al*.,1972) .

LDL–C = Total cholesterol –(TG/5)+ HDL-C)

3-7-5: Measurement of serum Very Low Density Lipoprotein-Cholesterol (VLDL-C)

Concentration of VLDL-C was determined according to method by Friewaled *et al* .,(1972), Tietz,(1999).

3-8: Hormonal Parameters Assessment

3-8-1: Determination of serum FSH, LH and PRL :

Procedure

Vidas (FSH ,LH and PRL) in human serum employed on the VIDAS family tools for the quantitative , using ELFA technique (enzyme Linked Fluorescent Assay), The method is used according to Biomerieux Kit procedure of Vidas FSH, LH and PRL (L'hermite,1976; Bardin and Paulsen , 1981).

1- Only remove the required reagent from the refrigerator and allow them to come to room temperature for 30 min
2- Use one (FSH,LH and PRL) strip and one SPR for each sample ,control or calibrator to be tesed .
3- The test is identified by the (FSH, LH and PRL) code on the instrument the calibrator must be identified by (S10 and tested in triplicate, it shoud be identified by C1 .
4- The calibrator control and sample test portion is 200µL .
5- Insert the (FSH,LH and PRL) SPR and (FSH,LHandPRL) strip into instrument .
6- Initiate the assay as directed in the user's manual all the assay steps are performed automatically by the instrument .
7- Reclose the vials and return them to2-8Ċ° .

8- The assay will be completed within approximately 40 minute after the assay is completed remove the SPR and strip from the instrument.
9- Dispose of the used SPR and strips into an appropriate recipient .

Expected value for men

LH	FSH	PRL
1.1-7.0 mlU/ml	**1.7-12.0 mlU/ml**	**3-25ng/ml**

3-8-2: Determination of Serum Testosterone

Procedure

Vidas testosterone (T) is an automated quantitative examination of total testosterone in human serum employed on the VIDAS family tools , using ELFA technique (Enzyme Linked Fluorescent Assay) , the method is used according to Biomerieux Kit procedure (Wheeler, 2006) .

1- Use one (TES2) strip and one (TES2) SPR for each sample , control or calibrator to be used .
2- The test is identified by the (TES2) code on the instrument ,the calibrator must be identified by (S) and tested ,it should be identified by C1.
3- If necessary , clarify sample by centrifugation.
4- The calibrator ,control and sample test portion is 100 µL.
5- Insert the (TES2)SPR and (TES2) strip into the instrument .
6- Initiate the assay as directed in the user's manual all the assay steps are performed automatically by the instrument .
7- Reclose the vials and return them to the required temperature after pipetting.

8- The assay will be completed within approximately 40 minute after the assay is completed remove the SPR and strip from the instrument.

9- Dispose of the used SPR and strips into an appropriate recipient.

Expected Values for men

Median (ng/ml)	**percentile 5% (ng/ml)**	**Percentile 95% (ng/ml)**
5.61	2.27	10.30

3-8-3:Determination of serum Estradiol (E2)

Procedure

Vidas Estradiol (E2) is an automated quantitative examination of total 17β–estradiol in human serum employed on the VIDAS family tools for the quantitative , using ELFA technique (Enzyme Linked Fluorescent assay) ,the methods is used according to Biomerieux Kit procedure (Lynskey *et al.*,1991).

1- Remove only the reagent from the refrigerator and allow them to come to room temperature for 30 min .
2- Use one (E2) strip and one SPR for each sample ,control or calibrator to be used .
3- The test is identified by the (E2) code on the instrument the calibrator must be identified by (S1) and tested in triplicate , it should be identified by C1.
4- The calibrator ,control and sample test portion is 200µL.
5- Insert the (E2) SPR and (E2) strip into the instrument .

6- Initiate the assay as directed in the user's manual all the assay steps are performed automatically by the instrument.
7- Reclose the vials and return them to2-8 °Ċ.
8- The assay will be completed within approximately 40 minute after the assay is completed remove the SPR and strip from the instrument.
9- Dispose of the used SPR and strips into an appropriate recipient

Men	**<62 pg/ml**

Expected value for men

3-9: Fertility parameters

3-9-1: Semen analysis

In the present study, semen analysis was done according to the World Health Organization standard criteria (2010).

Containers which contain semen were placed in an incubator at 37°C allowed for liquefaction (Nafa and Eshre, 2002). The liquefied semen was accurately mixed by glass Pasteur pipette for few seconds, after that the sample was checked in specifics by macroscopic and microscopic screening.

3-9-2: Macroscopic examination

3-9-2-1: Liquefaction

After ejaculation,semen usually looks like a jelly coagulated mass. It will liquefy in only some minutes at lab temperature. Since continuing of liquefaction,the seminal fluid turns into extra standardized, the whole sample generally liquefies within 15minutes at room temperature, although it may not often raise to 60 minutes or further. (WHO, 2010):

3-9-2-2: Semen appearance:

Normal liquefied semen was homogeneous light grey or grey-white, opalescent, and opaque. Any other appearance was considered as abnormal. If the color may be unusual red brown, following the presence of red blood cells in the ejaculate (Haemospermia), otherwise yellow in patients taking some vitamins or drugs or with jaundice (WHO,2010).

3-9-2-3: Semen volume:

Semen volume was estimated by suction the specimen from container into a graduated plastic disposal pipette. Plastic syringe should not be used because they may affect sperm motility and hypodermic needles are unsafe. (WHO, 2010):

3-9-2-4: Semen viscosity:

Following liquefaction, sometimes the viscosity referred to like ((constancy)). According to WHO 2010 manual. The sample was evaluated through smoothly aspirating it into a broad-bore (about 1.5 mm diameter) plastic disposable pipette, the semen was allowed to drop by gravity and the length of the thread was observed.

A standard sample was put down from the pipette in small distinct drops. Condition viscosity is abnormal; the drop will be like a thread longer than 2 cm in long. Otherwise, the evaluation of the viscosity can be done by putting a glass rod into the sample, and then the length of the thread that forms upon withdrawal of the rod was observed. The viscosity should be reported as unusual when the thread overtake 2 cm (WHO, 1999 b).

3-9-2-5: Semen pH:

The pH of prostatic secretion seminal fluid represents the stability among the values of pH of the diverse accessory gland productions generally, the acidic and the alkaline seminal vesicular secretion(WHO, 2010).

Fresh liquefied semen was mixed well in their original container; this was accomplished by carefully aspirating the specimen a few times into a large bore (about 1.5mm diameter) of pipette with attention in order to prevent air bubbles forming. Aliquot 10µL of semen were removed and placed onto a clean pre-warm glass slide immediately after mixing and covered with 22×22mm warm cover-slip.

All semen samples have been remixed before taking replicate aliquots and the results of these replicates must not show significant differences to be agreed.

3-9-3: Microscopic examination

3-9-3-1: Semen concentration:

Undiluted wet preparation of a liquefied semen was made, spermatozoa in 10 random microscopic fields were watchfully counted under×40 high power field (HPF). Sperm concentration is calculated from the mean of these 10 randomly chosen fields multiplied by 10^6 More than a replicate was examined for samples with low sperm number, in which entire cover-slip scanned continuously in a zigzag fashion. Moreover, a total spermatozoa number in the ejaculates were examined through the concentration of the spermatozoa per 1ml multiplied by the sample volume (Uni and Björndahl, 2002).

3-9-3-2: Sperm motility

A wet preparation was prepared as described previously, then the slide was examined after waiting for a few seconds to get rid of current, 40× magnification power for examination systematically scanned slide fields chosen randomly in an area far from the cover slip edge by approximately 5mm to avoid drying effect which could decrease sperm motility. About 200 spermatozoa in a total of at least 10 fields was counted and categorized in compliance to WHO simple system in grading motility for 2010, categorization was recorded as sets:

- Progressive motility (PR): Spermatozoa moving actively, either linearly or in a large circle, regardless of speed.
- Non-progressive motility (NP): All other patterns of motility with an absence of progression, e.g. swimming in small circles, the flagella force hardly displacing the head, or when only a flagella beat can be observed.
- Sluggish, Slow motile
- Immotility (IM): No movement(dead)

Collectively, PR, NP and IM of spermatozoa in random ten fields have been counted and the percentages of each grade mean respectively was calculated. Taking this into consideration, those percentages should be added but not up to 100%. The lesser reference limit for total motility (PR +NP) is 40%, and for progressive motility (PR) is 32% (WHO, 2010).

3-9-3-3 Sperm morphology%:

Actually, normal sperm morphology was assessed directly after the counting of sperms observed in a wet preparation under 40x. 200 sperms were counted in 10 random fields and normal sperm morphology

expressed as percentage calculated by dividing the number of morphologically normal/ total count of sperm 200.

The reference value for morphologically normal forms ≥ 30% that has been elucidated in third edition of the WHO manual (1999 b) was dependent as a lower reference in the present study.

3-9-4: Seminal plasma preparation and storage

Centrifugation of the semen samples for 15 minutes at 2600 rpm. Cautiously recovering the supernatant seminal plasma was rapidly and place to freeze at -20° C to be measured biochemical parameters in seminal plasma (WHO, 2010):

3-9-5: Biochemical parameters in Seminal plasma

3-9-5-1: Fructose

Principle

D –fructose , in presence of ATP ,is transformed from Esochinase (EK) in fructose -6 phosphate. The fructose -6 phosphate is transformed from phospho-Gluco-isomerase (PGI) in glucose -6 phosphate , that in its turn is transformed in 6-phosphgluconate from G6P-DH with formation of NADPH. NADPH formed in this reaction causes an increas of absorbance at 340 nm. The principle of the kit is according (Beutler, 1984).

Reagent

Buffer	Buffer of good >10 mM pH 7.5 ;NADP >0.2 mM
Substratum /Enzyme	ATP> 2 mM ; HK > 10 U/L
Starter 1	G6PDH> 5 U/L

Starter 2	PGI > 50 U/L
Standered	Fructose 1 mg /ml
Diluent	Sample detergent

Sample preparation

Centrifuge sample at 3000 rpm for 10 min. Diluent 10 µl of sample with 600 µl of diluent.

Procedure

Reagent	**Blank**	**Standard**	**Sample**
Substratum reconst.	1000 µl	1000 µl	1000 µl
Standard	--	20 µl	--
Sample	--	--	20 µl
Distilied water	20 µl	--	--
Starter 1	25 µl	25 µl	25 µl
Mix, wait the end of reaction (10 min)and measure absorbance of solation (A_1) against blank			
Starter 2	25 µl	25 µl	25 µl
Mix , wait the end of reaction (10 min) and measure absorbance osf solution against blank			

Calculation

A_2(sample)-A_1(sample)/ A_2(standard)-A_1(standard)* 61*1

Expected value

Fructose (mg/ml) 2-5

3-9-5-2: Zinc

Principle

The zinc, at room temperature reacts with cromogen present in the reagent giving a coloured complex that have a strenth proportional to zinc concentration present in the sample. The principle of the kit is according (Makino,1991).

Reagent

Reagent A	Boron buffer 0.37 M pH 8.2; Saliciladoxima 12.5 mM; Dimetilglioxima 1.25 mM; Tensioactive and conservative.
Reagent B	NITRO-PAPS; 0.4 mM conservative
Diluent	Solution of sample dilution
Standard	Zinc ion 2 µg/ml, stabilizer and conservative

Sample preparation

Centrifuge the sample at 3000 rpm for 10 min and dilute supernatant 1;1000 with diluent.

Procedure

Reagent	**Blank**	**standard**	**Sample**
Distililled	50 µl	--	--
Standard	--	50 µl	--
Sample	--	--	50 µl
Work reagent	1 ml	1 ml	1 ml

Mix and after 5 min read absorbance against blank at 578 nm . the color stable for 30 Min .

Calculation

Zinc µg/ml = A (sample)/A(standard)*2*200

Expected values

200-350 µg/ml

3-9-5-3: Alkaline phosphatase(ALP)

Principle

This kit uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay Human alkaline phosphatase(ALP). Add alkaline phosphatase(ALP) to wells that are pre-coated with alkaline phosphatase(ALP) monoclonal antibody and then incubate. After incubation, add anti ALP antibodies labeled with biotin to unite with streptavidin-HRP, which forms the immune complex. Remove unbound enzymes after incubation and washing, then add substrate A and B. The solution will turn blue and change to yellow with the effect of acid. The shades of solution and the concentration of Human alkaline phosphatase(ALP) are positively correlated.

a) Hold kit at room temperature for at least 30 minutes once removed from 2-8℃ environment. Coated ELISA plates should be stored in sealed bag if not used after opening.
b) When adding samples, sample injector must be used each time and should also be frequently checked for precision to avoid individual error.
c) The operating instructions must be strictly followed and the reading of ELISA reader must be set as the standard for determining the experiment result.
d) Pipette tips and seal plate membrane in hand should not be used more than once in order to avoid cross contamination.
e) All samples, washing concentration, and all waste should be disposed as infective agents.

f) Other reagents not needed shall be packed or covered. Reagents of different batches shall not be mixed and should be used before their respective validity dates. Substrate B is sensitive to light and therefore should not be over-exposed to light.

Assay procedure

a) Dilution of standard solutions: (This kit provides one standard original concentration. Users may independently dilute in small tubes following the chart below:

320IU/L	Standard No.5	120µl Original Standard + 120µl Standard diluents
160IU/L	Standard No.4	120µl Standard No.5 + 120µl Standard diluents
80IU/L	Standard No.3	120µl Standard No.4 + 120µl Standard diluents
40IU/L	Standard No.2	120µl Standard No.3 + 120µl Standard diluents
20IU/L	Standard No.1	120µl Standard No.2 + 120µl Standard diluents

Standard solutionNo.5No.4 No.3No.2No.1

b) The number of stripes needed is determined by samples to be tested added by the standards. It is recommended that each standard solution and each blank well be arranged with multiple wells as much as possible.

c) Sample injection: 1) Blank well: Do not add sample, anti ALP antibody labeled with biotin and streptavidin-HRP; add chromogen

reagent A & B and stop solution, each other step operation is the same. 2) Standard solution well: Add 50µl standard and streptomycin-HRP 50µl (biotin antibodies have united in advance in the standard so no biotin antibodies are added). 3) Sample well to be tested: Add 40µl sample and then 10µl ALP antibodies, 50µl streptavidin-HRP. Then cover it with seal plate membrane. Shake gently to mix. Incubate at 37℃ for 60 minutes.

d) Preparation of washing solution: Dilute the washing concentration (30X) with distilled water for later use.
e) Washing: carefully remove the seal plate membrane, drain liquid and shake off the remainder. Fill each well with washing solution, let stand for 30 seconds, then drain. Repeat this procedure five times then blot the plate.
f) Color development: First add 50µl chromogen reagent A to each well, and then add 50µl chromogen reagent B to each well. Shake gently to mix. Incubate for 10 minutes at 37℃ away from light for color development.
g) Stop: Add 50µl Stop Solution to each well to stop the reaction (color changes from blue to yellow immediately at that moment).
h) Assay: Take blank well as zero, measure the absorbance (OD) of each well one by one under 450 nm wavelength, which should be conducted within10 minutes after having added stop solution.
i) According to standards concentrations and corresponding OD values, calculate the linear regression equation of the standard curve. Then according to the OD value of samples, calculate the concentration of the corresponding sample. Statistical software could also be employed.

3-9-5-4: Glutathione(GSH)

Principle

This kit uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay Human glutathione (GSH). Add glutathione (GSH) to wells that are pre-coated with glutathione (GSH) monoclonal antibody and then incubate. After incubation, add anti GSH antibodies labeled with biotin to unite with streptavidin-HRP, which forms the immune complex. Remove unbound enzymes after incubation and washing, then add substrate A and B. The solution will turn blue and change to yellow with the effect of acid. The shades of solution and the concentration of Humanglutathione(GSH) are positively correlated.

Assay procedure

a) Dilution of standard solutions: (This kit provides one standard original concentration. Users may independently dilute in small tubes following the chart below:

24ng/ml	Standard No.5	120µl Original Standard + 120µl Standard diluents
12ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard diluents
6ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard diluents
3ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard diluents
1.5ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard diluents

Standard solutionNo.5No.4 No.3No.2No.1

b) The number of stripes needed is determined by that of samples to be tested added by the standards. It is recommended that each standard solution and each blank well be arranged with multiple wells as much as possible.

c) Sample injection: 1) Blank well: Do not add sample, anti GSH antibody labeled with biotin and streptavidin-HRP; add chromogen reagent A & B and stop solution, each other step operation is the same. 2) Standard solution well: Add 50µl standard and streptomycin-HRP 50µl (biotin antibodies have united in advance in thestandard so no biotin antibodies are added). 3) Sample well to be tested: Add 40µl sample and then 10µl GSH antibodies, 50µl streptavidin-HRP. Then cover it with seal plate membrane. Shake gently to mix. Incubate at 37℃ for 60 minutes.

d) Preparation of washing solution: Dilute the washing concentration (30X) with distilled water for later use.

e) Washing: carefully remove the seal plate membrane, drain liquid and shake off the remainder. Fill each well with washing solution, let stand for 30 seconds, then drain. Repeat this procedure five times then blot the plate.

f) Color development: First add 50µl chromogen reagent A to each well, and then add 50µl chromogen reagent B to each well. Shake

gently to mix. Incubate for 10 minutes at 37℃ away from light for color development.

g) Stop: Add 50µl Stop Solution to each well to stop the reaction (color changes from blue to yellow immediately at that moment).

h) Assay: Take blank well as zero, measure the absorbance (OD) of each well one by one under 450 nm wavelength, which should be conducted within10 minutes after having added stop solution.

i) According to standards concentrations and corresponding OD values, calculate the linear regression equation of the standard curve. Then according to the OD value of samples, calculate the concentration of the corresponding sample. Statistical software could also be employed.

3-10: Statistical Analysis

Data were analyzed by one way ANOVA by general liner model procedure using statistical package for social science (SPSS) version 22. The comparisons between means scores were made using least significant differences (LSD) using Genstat3statistic program. The difference were considered to be significant at $P<0.05$ using multivariate model in SPSS. The data are presented as mean ± S.E. (SPSS, 1998).

Chapter Two

Literature Review

2-1: Diabetes mellitus (DM)

Diabetes mellitus is a combination of heterogeneous disorders commonly presenting with episodes of hyperglycemia and glucose intolerance, as a result of lack of insulin , defective insulin action, or both (Sicree *et al.*, 2006).

Diabetes mellitus produces when the defect occurs in the insulin secretion, insulin action, or both. In the physiologic state the pancreatic β- cells normally respond to hyperglycemia by producing insulin, which is a hormone allows the cells to use glucose and prevents the conversion of glycogen to glucose in the liver **(**Devendra *et al.*, 2004).

The effect of insulin is to decrease the level of blood glucose, disturbance of insulin function may occur either because of β – cells are unable to produce sufficient insulin, or because tissue response to the secreted insulin is impaired. The end result is raised the level of blood glucose (Hyperglycemia) (Preshaw and Taylor, 2011). General symptoms of marked hyperglycemia include weight loss, polydipsia, polyphagia, and polyuria are more frequent in type I than type II DM (Cooke and Plothick, 2008). The global prevalence of diabetes mellitus in 2010 is 285 million cases and this is projected to be 439 million in 2030 (Shaw *et al*., 2010).

2-2: Classification of diabetes mellitus

There are numerous types of diabetes mellitus presently, the World Health Organization (WHO) and the American Diabetes Association (ADA) classify these types according to the etiology of the disease, in: Type I diabetes mellitus (T I DM), type II diabetes mellitus (T II DM) and gestational diabetes mellitus (GDM) (Araujo *et al.*, 2014**).**

2-2-1: Type I Diabetes Mellitus

Type I DM insulin dependent Diabetes mellitus (IDDM)is a confusion described by autoimmune destruction for insulin- producing from pancreatic β cells, culminating insulinopenia with resultant hyperglycemia, which is one of the commonest chronic disorders. It is diagnosed during infancy requiring exogenous insulin for survival, often the loss of β- cells begins in infancy and continues for several months or years. Diagnosed the type I DM may occur when 80 % of the β- cells have been destroyed (Zipitis and Akobeng , 2008).

2-2-2: Type II diabetes mellitus

Type II diabetes called Non Insulin dependent diabetes mellitus(NIDDM) is a complex disorder characterized by imbalance between insulin resistance and insulin secretion that induce liver glucose output by preventing glycogen formation and stimulating glycogenolysis and gluconeogenesis then excess rates of liver glucose production result in the development of overt diabetes mellitus, especially fasting plasma glucose (Michael *et al.*, 2000; Holt and Hanley, 2012). The diagnosis of type II DM occurs in younger adults, even during childhood (IDF, 2009). However , type II DM is being increasingly seen in the teenager years and occurs without loss of β islet cells function but the peripheral tissue is resisted to insulin action, a disorder in which the cells do not use insulin correctly, as the necessary for insulin increases. The pancreas gradually loses its ability to produce insulin (Defronzo *et al.*,2004; Cohen, 2006).

2-2-3: Gestational diabetes mellitus (GDM)

In addition to types I and II, a third type of diabetes is existed. Its affects only women pregnancy and is called gestational diabetes mellitus (GDM), that is a temporary type of diabetes that occurs during pregnan-

cy. Most women with GDM will return to normal glucose levels after delivery (Smith-Morris, 2005). GDM presents in women which makes a higher risk for the development of T2DM in later years of life. Newborns of mothers with GDM are increased risk for acute perinatal complications including hypoglycemia and jaundice fetus , the human chorionic somato tropin (HCS) acts together with growth hormone (GH) from the mother's pituitary to produce diabetic-like effect in pregnant women(Vandana *et al*., 2015).

These hormones promote: (1) lipolysis and increased plasma fatty acid concentration. (2) Glucose-sparing by maternal tissues and therefore increased blood glucose concentrations. (3) Polyuria (excretion of large volume of urine), thereby producing a degree of dehydration and thirst. This diabetic-like effect in mother helps to ensure a sufficient supply of glucose for the plaque and fetus (Fox, 2003).

2-2-4: Other specific type

A number of other types of diabetes exist in. A person may exhibit characteristics of more than one type. For example, in latent autoimmune diabetes in adults (LADA), also called type 1.5 diabetes or double diabetes, people show signs of both type 1 and type 2 diabetes(ESC, 2013). Diagnosis usually occurs after age of 30 years. Most people with LADA still produce their own insulin when first diagnosed, like those with T2DM, but within a few years, they must take insulin to control blood glucose levels. In LADA, as in T1DM, the B-cells of the pancreas stop making insulin because the body's immune system attacks and destroys them. Some experts believe that LADA is slowly developing kind of T2DM (NIH, 2008).

Other etiologies for specific types are presently less common causes of diabetes mellitus but are those in which the underlying defect or disease process, Such as genetic (Genetic defects of ß-cell function, genetic defects in insulin action), diseases of the exocrine pancreas (pancreatitis, trauma/pancreatectomy, cystic fibrosis) , endocrinopathies (acromegaly, glucagonoma, hyperthyroidism), drug or chemical induced (vacor, pentamidine, nicotinic acid) , infections (congenital rubella, cytomegalovirus) (WHO, 1999 a)

2-3: Diagnosis of diabetes mellitus

Diabetes mellitus is a complex group of diseases with a variety causes, people with diabetes have high blood glucose, also called high blood sugar or hyperglycemia (Rother, 2007). The clinical diagnosis of diabetes is often prompted by symptoms such as increased thirst and urine volume, recurrent infections, unexplained weight loss and, in severe cases, drowsiness and coma, high levels of glycosuria are usually present (WHO, 1999 a). In the absence of symptoms above, the following criteria should be applied to confirm the presence of diabetes. The diagnosis of diabetes is shown in table (2-1)

Table (2-1):Blood glucose levels for diagnosis of diabetes

Classification	Fasting blood glucose (FBG)	Oral Glucose Tolerance Test (GTT)	HbA1c%
Non diabetes	65-99 mg/dl	≤ 140 mg / dl	4.1 – 5.6%
Prediabetes	100-125mg/dl	140-199mg /dl	5.7 – 6.4%
Diabetes type2	≥ 125 mg/dl	≥ 200	≥ 6.5%

2-4: Glycated HemoglobinA1c(HbA1C)

Glycated hemoglobin is a modified hemoglobin with a stable adduct of glucose to the N- terminal valine of beta chain. It is the gold standard

to monitor the control of the blood glucose in patients with diabetes mellitus. The patient's measurement of hemoglobin A1C provides a picture of long-term glycemic control and reflects average glycemic control over the previous 8 – 12 weeks . It is formed in a non-enzymatic pathway by hemoglobin's normal exposure to high plasma levels of glucose. The use of this test has been extended to diagnose and screen for diabetes mellitus with the endorsement of several international diabetes societies and the World Health Organization. In2010, the International Expert Committee and the American Diabetes Association proposed diagnostic criteria for diabetes and pre-diabetes based on HbA1c levels. These are HbA1c ≥6.5% to diagnose diabetes mellitus and between 5.7–6.4% for pre-diabetes (ADA., 2013). The glycated hemoglobin has been used as a biomarker of long-term glycemic control (Goldenburg and Punthakee , 2013). HbA1c values between 6% - 7% were considered as a sign of good control of diabetes, HbA1c values between 7.1% -8% indicated moderated control, and HbA1c value >8% were designated as poor control of diabetes (Marshall, 2010).

HbA1c is considered the golden standard in the management of type 2 diabetes mellitus (T2DM). It is part of the glycemic objectives and mentioned in all the guidelines and clinical recommendations for the management of T2DM, also increased HbA1c values are associated with increased risk of T2DM specific complications (ADA, 2013).

2-5: Lipid profile

2-5-1: Total Cholesterol(TC)

Cholesterol could be a steroid lipid, found in the plasma membrane of all body tissue and transported within the plasma of all animals, its bi-

osynthesized by the internal organ tissue from the acetyl-CoA from carboxylic acid chemical reaction and its very important for the conventional functioning of the body (Miller and Bose, 2011). Cholesterol is an important element of the plasma membrane providing stability, it makes the membrane fluidness stable over a bigger temperature interval, it's the major precursor for the synthesis vitamin D and steroid hormones. Furthermore the recent research shows that sterol has an important role for the brain biological process, also as within the immune system including protecting against cancer (John *et al*., 2002)

2-5-2: Triglycerides (TG)

Triglyceride (TG) are neutral fats found within blood stream and fat tissue, they are derived from dietary lipids or made within the body from alternative metabolites like carbohydrates, triglyceride are major parts of very low density lipoprotein and chylomicrons. Excess triglyceride in plasma is as hypertriglyceridemia. Its joined to arteriosclerosis by extension the danger of cardiopathy and stork (Schwarz *et al* ., 2003)

2-5-3: High Density Lipoprotein (HDL-C)

High density lipoprotein (HDL) are usually called good cholesterol. They take away excess sterol from tissue and vessel walls and carry it to the liver wherever its far from blood and discarded. The quantity of serum HDL is invers elyrelated with the incidence of cardiovascular disease, the higher level of HDL cause the lower risk of coronary artery disease and vice versa.HDL carries cholesterol within the blood from different elements of the bod back to the liver for excretion. So HDL helps keep sterol from increase within the walls of the arteries (Kwiterovich, 2000).

2-5-4 Low Density Lipoprotein (LDL-C)

Low density liopoprotein called bad cholesterol, they are maked primarily by the breakdown of VLDL. They contain very little triglyceride and big quantity of cholesterol (60-70% of total cholesterol). Although the particles are abundant smaller than chylomicron and VLDL, low density lipoprotein particles can vary in size and chemical structure , LDL carries most of the cholesterol within the blood. The additional low density lipoproteins within the blood , the larger risk of cardiopathy (Sinderman *et al*., 2003).

2-5-5 Very low Density Lipoprotein (VLDL)

VLDL is made in the liver by the combination of cholesterol, triglycerides and apoprotein. VLDL transports endogenous triglycerides, phospholipids, cholesterol and cholesteryl esters. It functions as the body's internal transport mechanism for lipids. These lipoprotein particles are smaller than chylomicrons and contain less triglyceride but contain significant amount of cholesterol (10-15% of cholesterol). As the VLDL circulates in the blood, triglycerides are deposited and the particles get smaller, eventually becoming teins (LDL). Serum with large mass of VLDL will be cloudy *(*Lowe and Howard,1995*)*.

2.6 Relationship between Diabetes Mellitus Type II and Lipid profile:

The principles abnormal of lipid metabolism in diabetes are acceleration of lipid catabolism, with increased formation of ketone bodies and decreased synthesis of fatty acid and triglycerides. Fifty percent of an ingested glucose load is normally burned to CO_2 and H_2O 5% is converted to glycogen and 30-40% converted to fat in fat depots. In diabetes less than 5% converted to fat even though the amount burned to

CO_2 and H_2 is also decreased and the amount converted glycogen is not increased. Therefore, glucose accumulates in the bloodstream and spills over into the urine (Ganong., 2003). It is play an important role in pancreatic cell responses (Yaney and Corkey., 2003).

Many studies mentioned that increase cholesterol in diabetes patients compared with control (Hashim,2015; Nema,2008). Also study by Al-Aridhi and Al-Ahmed. (2015), where found increase significant of cholesterol and triglyceride. In study by Dixit *et al*., (2014) found of HDL which decrease significantly compared to control. In other hand study by Singh *et al*., (2015) noted high level of LDL in patients type II of diabetes when compared with control.

2-7: Hormones

2-7-1: Follicular stimulating hormone (FSH)

Follicular stimulating hormone a gonadotropin, a glycoprotein,it is synthesized and secreted by the gonadtropic cells of the anterior pituitary gland and regulates the development, growth, pubertal maturation, and generative processes of the body. Follicle-stimulating hormone and luteinizing hormone work along within the male and female genital system (Fan and Hendrickson, 2005).

2-7-2: Luteinizing hormone (LH)

Luteinizing hormone which made by gonadotropin cells within the anterior pituitary gland. In males, wherever luteinizing hormone had also been referred to as interstitial cell–stimulating hormone (ICSH), it stimulates leydig cell production of testosterone(Kodaman and Behrman, 2001).

The secretion of LH and FSH are essential for spermatogenesis as well as production and maintenance of testicular sex hormones, FSH stimulates the proliferation and differentiation of prepuberal sertoli cells during neonatal stage. Also During puberty FSH mediate the maturation of sertoli cells into functional cells. In adult males, FSH indirectly sustains spermatogenesis by controlling an array of metabolic functions. As for LH, its action is on the leydig cells and allows for production of testosterone (Sharpe *et al.*, 2003; Huhtaniemi, 2010).

2-7-3: Total Testosterone(TT)

Testosterone is a steroid hormone derived from cholesterol and synthesized by the leydig cell in large amounts in male testes, and in less amounts by adrenal glands and female ovaries (Mazur and Booth, 1998; Eisenegger *et al*., 2011).

Testosterone secretion is regulated by feedback of testosterone on the discharge of gonadtropic hormone from the pituitary gland. Testosterone is very protein-bound. In males, ninety-eight of the testosterone in circulation is bound; the worth is slightly lower in females. The bulk of the steroid is bound to a particular binding protein, generally remarked as sex hormone binding globulin (SHBG)or androgen binding simple protein, and to albumen (Dunn *et al*., 1981). Testosterone forms part of a class of hormones known as androgens. It is responsible for the maintenance of libido and masculine characteristics, stimulates muscle and bone growth and plays a vital role in the development of male reproductive organs (penis, prostate and scrotum) (Serra *et al*., 2013). Testosterone is considered to be the main androgen responsible for male fertility and spermatogenesis (Wang *et al*., 2009; Smith and Walker, 2014).

2-7-4: Estradiol

Estradiol is an important sex hormone in both males and females. It plays a vital role in development and maintenance of the fertility and the reproductive system (Nilsson *et al*., 2001; Carreau *et al*., 2008). In females, estradiol is mainly produced by the ovary and placenta and in males, by the testes and in both sexes by the adrenal cortex. Estradiol normally circulated bound to sex hormone binding globulin (SHBG) (Kahn *et al*., 2002; Wildman *et al*., 2013).

Estradiol forms part of a hormone estrogen and like other steroid hormones it is derived from cholesterol. During reproductive years in both sexes, most estradiol is produced by aromatization of testosterone from adrostenedione then converted to estrone, estrone in turn gets converted to estradiol. The enzyme responsible for this cascade 12 of conversions is called aromatase (Salway, 2004; Lehninger, 2005; Antal *et al*., 2008).

Studies were done by Nilsson *et al*. (2001) have demonstrated that estradiol yield an array of biological effects in the musculoskeletal, cardiovascular, immune and central nervous systems. Estradiol has also been observed to have an important role in preventing pathological processes of various tissues of the reproductive system (Prins and Korach, 2008; Lazari *et al*., 2009).

2-7-5: Prolactin (PRL)

Prolactin is a polypeptide hormone that is synthesized and secreted from specialized cells of the anterior pituitary gland, its protein that is best known for role in enabling females to produce milk (Freeman *et al*., 2000). It is synthesized in many extra pituitary tissue , among these sites are the cell of immune system such as macrophage , natural killer

cell , T and B lymphocyte, decidua, myometrium, breast, and prostate(Gerlo *et al*., 2006;Oakes *et al*., 2008)..

PRL plays an essential role in metabolism, regulation of the immune system and pancreatic development (Montgomery,2001).

Elevated levels of PRL decrease the levels of sex hormones estrogen in women and testosterone in men (Majumdar and Mangal, 2013). PRL within the normal reference ranges can act as a weak gonadotropin, but at the same time suppresses GnRH secretion (Grattan *et al., 2007).*

Physiologic levels of PRL in males enhance luteinizing hormone-receptors in Leydig cells, resulting in testosterone secretion, which leads to spermatogenesis (Hair et al., 2002). In men, excessive PRL is associated with gynecomastia and impotence (Guyton and Hall, 2011).

2-8: Relationship between diabetes mellitus type II and reproductive hormones

Male germ cell is dependent on the balanced endocrine of hypothalamus pituitary and the testis. Gonadotropin releasing hormone (GnRh) secreted by the hypothalamus stimulate the release of gonadotropins FSH and LH from the pituitary gland FSH binds by receptors in the sertoli cells and stimulates spermatogenesis LH stimulates the production of testosterone in Leydig cells, which in turn may act on the Sertoli and per tubular cells of the seminiferous tubules and stimulates spermatogenesis (Ganong, 2003 and Mohieldin *et al.*,2016).

Several studies confirm the effect of diabetes mellitus type II on reproductive hormones.

AL-Aaraji,(2016), mentioned testosterone decrease and estradiol increase significant with compared control.

Hussein and Al-Qaisi (2012) study Effect of Diabetes mellitus Type 2 on Pituitary Gland Hormones (FSH, LH) in Men and Women in Iraq mentioned to decrease of FSH and LH when compared to control .

Also Onah, *et al*,.(2013) Found increase significant of prolactin level in diabetic mellitus when comapared with control.

2-9: Male fertility

Fertility is the natural capability of producing offspring's. As a measure, "fertility rate" is the number of children born per couple, person or population. The primary reproductive organs of the male are the testes, or male gonads, which have both an exocrine (sperm producing) function and an endocrine (testosterone producing) function. The accessory reproductive structures are ducts or glands that aid in the delivery of sperm to the body exterior or to the female reproductive tract.(El-Refi ,2013)

2-9-1: Structure of the testis

The testis consists of convoluted seminiferous tubules embedded in a very connective tissue matrix that known as interstitium (Figure 2.1). The interstitium contains mixture of blood and lymph vessels, nerves, formative cell , macrophages and Leydig cells. The epithelial tissue of seminiferous tubules consists of frequently dividing germ cells that manufacture spermatozoon cells and supporting Sertoli cells. It pass from the seminiferous tubules to the testis, set within the bodily cavity testis, so to the epididymis wherever newly-created spermatozoon to mature. The sperm cell entrance the vas deferens, and are exit through the urethra (Krohmer, 2004).

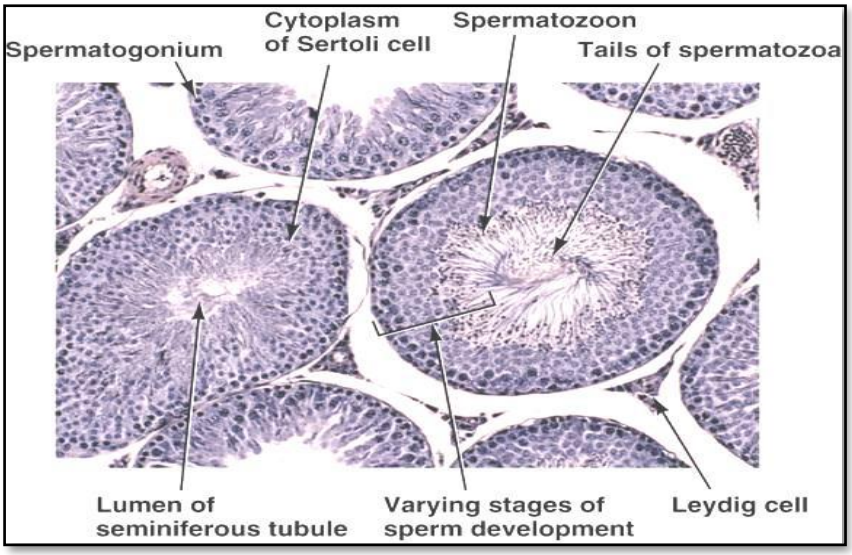


Figure (2-1) Cross- section of the testis showing sperm producing tube (seminiferous tubule) and leydig cell (Ohl *et al.*, 1996).

2-9-2: Spermatozooa

A spermatozoon or spermatozoon (spermatozoa), from the ancient Greek σπέρμα (seed) and ζον (living being) and more commonly known as a sperm cell, is the haploid cell that is the maleῷ gamete (Scanlon and Sanders, 2011).

Sperms are highly specialized, differentiated and condensed cells that do not divide. Approximately 60 μm long and 1 μm wide, each sperm is composed of the head, mid piece (body), and tail (Karpenko *et al.*, 2007).

The sperm head is composed of a nucleus, in which the deoxyribonucleic acid (DNA) condensing center and linker histones have been incompletely replaced during spermiogenesis by protamines, (Brewer *et al.*, 2002; Dadoune *et al.*, 2004). In addition to the mid piece and the tail, the sperm can be divided into four major segments include:

1. Connecting piece: is composed of nine striated or segmented columns (Toyooka *et al.*, 2003).

2. Mid piece: is covered by the mitochondrial sheath in form of a helix of approximately 75–100 sperm mitochondria, which generate the energy for the sperm flagellar motility. Each sperm mitochondrion carries multiple copies of the paternal mitochondrial genome. **(**Sutovsky *et al*., 2004).
3. Principal piece: The principal piece is enclosed by the protective scaffold of fibrous sheath. (Eddy *et al*., 2003).
4. End piece: contains axonemal doublets and the ends of outer dense fibers and fibrous sheath.(figure 2-2)

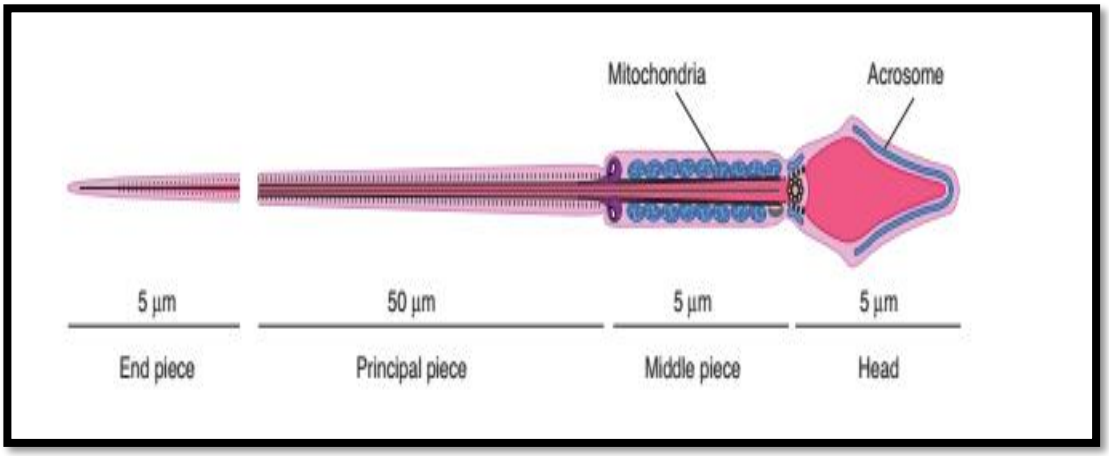


Figure (2-2) : Human spermatozoa (Ganong, 2003)

2-9-3: Effect of glucose on sperm

Sperm cell is the most differentiated mammalian cell. The main goal of sperm consists of transferring male haploid DNA to female DNA through a series of mechanisms that imply their displacement along the female genital tract and fertilizing ability(Rodriguez and Martinez,2007) Energy in sperm cells is mainly used to maintain the motility to complete capacitation and subsequent acrosome reaction. (Flesch and Gadella,2000).

Also, need energy to acquire and maintain motion competence after epididymal maturation because they are actually immotile in testis. Much adenosine triphosphate (ATP) in sperms is consumed for maintaining the motility, except some metabolites, such as lactate and citrate, sperm mainly utilize sugars as an energy fuel including glucose, mannose, and fructose (Yanagimachi,1994).

2-9-4: Seminal Fluid components

Semen is composed from sequential secretions from several male glands, such as natural α –glycosidase, hyaluronidas, carnitine, glycerol-phosphocholine, fructose, prostaglandin, citrate, zinc, selenium, prostate specific antigen, mucus, that secreted from accessory gland such as, cowpers glands, prostate and seminal vesicles, each fraction accounting for approximately 5%, 15-30% and 60% of the total ejaculate, respectively (Björndahl and Kvist, 2003). The total number of spermatozoa in the ejaculate and the secretory activity of the glands can estimate spermatozoa production by ejaculate´s total volume, which influences spermatozoa quality (WHO, 2010). According to recommendations of the World Health Organization´s (WHO), (2010) Seminal fluid has been considered as just a vehicle for sperm delivery to fertilize the oocyte. However, in the last years several studies suggested that this body fluid consists of important bioactive signaling agents that evoke cellular and gene expression changes in the female immune system (Robertson and Sharkey, 2016).

Several parameters are analyzed during conventional semen analysis. Spermatozoa concentration and total spermatozoa count as well as motility and morphology are the most important assessments. Total volume, pH, viscosity, appearance, as well as the presence of non-sperm

cells represent the secretory activity of the glands and reveal the presence of a concomitant infection (WHO, 2010).

2-9-4-1 Fructose

Fructose is vital to the practical integrity of spermatozoa it is that the major supply of glycolytic energy so as to keep up motility (WHO, 1992). The reference worth for traditional concentrations of fructose is predicated on the studies by Cooper *et al*, (1991) is 13µmol (2.34mg) or a lot of per ejaculate. Determination of the concentration of the saccharide is usually used in laboratories for a range of functions, together with the auxiliary diagnosing of retrograde ejaculation, clogging and nonobstructive azoospermia (Lu and Chen, 2007) and as a marker to assess seminal vesicular perform (Gonzales, 2001; WHO, 2010). High values of fructose have been individual with diabetes, oligozoospermia and azoospermia (Gonzales, 1994), while low values fructose detected in ejaculate with high density sperm with motile sperm, so that fructoslysis decrease concentration of fructose (Mauss *et-al.*,1974)

2-9-4-2 Zinc (Zn)

Zinc (Zn) is the second most abundant trace element in human, which can't stored in body and role of zinc in men health and its potential in germination quality of sperm and fertilization (Fallh *et al*., 2018)

Zinc is present in high concentrations in the seminal fluid, and it could play a multifaceted role in sperm functional properties. It influences the fluidity of lipids and, thus, the stability of biological membrane, it affects the stability of sperm chromatin, it is involved in the formation of free oxygen radicals and it could play a regulatory role in the process of capacitation and the acrosome reaction (Zhao *et al.*,2016). Zinc is a

structural part of key anti-oxidant enzymes such as superoxide dismutase (SOD) and Zinc deficiency impair their synthesis, leading to increased oxidative stress (Toma *et al*.,2013)

Reduction of Zn seminal plasma concentration leading to Idequate intake, reduced absorption, raised losses and redoubled demand during reproduction, Zn has various necessary functions, and its essential for conception, implantation and favorable pregnancy outcome(Foresta, 2014; De and Lamothe, 2010)

In study by Ghasemi *et al.* (2015)Seminal plasma zinc and magnesium levels and their relation to spermatozoa parameters in semen of diabetic men, found a significantly lower zinc level in seminal plasma of the diabetic group in comparison with the nondiabetic controls.

2-9-4-3 Alkaline phosphatase(ALP)

Alkaline phosphatase an enzyme that catalyzes the hydrolysis of organic phosphate at basic pH values, has been reported in the semen of men and animals, its secreted by prostate (Lewis- Jones *et a*l., 1992). The highest level of semen ALP activities are found in boars and also the lowest are found in humans (Jitendra *et al*., 2015).

ALP originates primarily from testis and epididymis and it may be used as a marker to differentiate azoospermia or oligspermia from ejaculatory failure in clinical cases (El-Bishbishy, *et al*.,2013). Suthager *et al,(*2009). mentioned alkaline phosphateas decrease significantly with diabetes induce rat.

2-9-4-4 Glutathione

One of the endogenous antioxidants are characterized as enzymatic antioxidants and are molecules generated by the seminal plasma

(Agarwal *et al*., 2005). The primary responsible of these antioxidants is the ability to neutralize and prevent the oxidation of biological molecules by ROS (Agarwal *et al*., 2005). Glutathione peroxidase (GPx) work with other molecules like glutathione reductase (GR) and glutathione(GSH) to achieve its action on Reactive oxygen species (ROS) (Choudhary *et al*., 2010). Oxidative stress happens when there is an overproduction of ROS or decrease deficiency of radical scavenger, such as glutathione (Bravi *et al.,* 2006). When ROS are produced in excess Superoxide dismutase (SOD) is the first antioxidant that acts to catalyse O2- into H2O2 and oxygen. Catlase (CAT) or GPx then comes and transforms H2O2 to H2O and O2 (Bahorun *et al*., 2006).

One major source of ROS in semen are sperm cell , from stages of development of male germ cell they able made small of ROS, the second source of ROS in semen are leukocyte , the high produce of ROS by leukocyte play an important role in the cellular defense mechanism against infection and inflammation (Walczak–Jedrzejowska *et al.*,2013).Many studies(Popool *et al*, 2017;de Oliveira *et al*, 2016;)which found GSH reduction significantly in diabetes rats when compared to control.

Conclusions

And

Recommendations

Conclusion

1. Dyslipidemia is over in patients with type 2 diabetes.
2. Gonadotropin (FSH,LH) and TT reduced in patients with diabetes type II and elevated of PRL and E2 levels leading to abnormal spermatogenesis .
3. Insufficient gonadotropin hormones in patients with diabetes mellitus type II caused a decreased of semen quality and a reduction in antioxidant agent and zinc element.
4. Decrement in antioxidant agent in seminal plasma (GSH) which playing an important role to removed the reactive oxygen species (ROS) .
5. Duration affected the patients with diabetes type II and playing a negative role on some fertility parameters.

Recommendation

1. Future studies with more number of sample and other parameters-such as another hormones , insulin resistance, trace element and antioxidant agent.
2. Future studies included prostatic components such as (citric acid, protelytic enzymes, acid phosphatase) in semen of patients diabetes .
3. Studying the relationship between obesity and fertility with related to diabetes.
4. Studying the sperm molecular basis in patients with diabetes mellitus .
5. Comparative study between semen composition and serum in T2DM.

Chapter Four

Results

4-1: Biochemical parameters in serum (According to age)

4-1-1: Fasting Blood Glucose (F.B.G) level

The value of F.B.G in third group (315.07±35.09 mg/dI) increased significantly (P< 0.05) in comparison with second group (253.53±13.46 mg/dI) and control group (93.20±2.13 mg/dI). Second group increased significantly (P< 0.05) comparison with control group. First group (285.46±17.15 mg/dI) increased significantly comparison with control group. No significant difference between third and first groups. No significant difference between second and first groups, Table (4-1).

4-1-2: HbA1C

The HbA1C value in the second group (11.75±0.55 %) was increased significantly (P <0.05) comparison the first (9.97±0.50 %) and third groups (9.06±0.48 %) and the control (5.00±0.15 %).There are no significant differences between first and third groups. First and third groups increased significantly in comparison with the control group, Table (4-1).

Table (4-1): The values of F.B.G and HbA1c in control and patient with DM T2 (According to age)

parameters / Groups	F.B.G **(mg/dl)**	**HbA1C (%)**
Control(30-59)	c 93.20±2.13	c 5.00±0.15
First group (30-39)year	ab 285.46±17.15	b 9.97±0.50
Second group (40-49)year	b 253.53±13.46	a 11.75±0.55
Third group (50-59)year	a 315.07±35.0.9	b 9.06±0.48
LSD	58.60	1.28

N=15
Values represented mean ± SE.

The different letters refer to significant difference among group at level of (P<0.05). The same letters refer to non-significant difference among group.

4-1-3: Lipid profile

4-1-3-1: Total Cholesterol (TC)

The values of cholesterol in the first group (244.20±15.08 mg/dI) increased significantly (P<0.05) in comparison with the second group (205.13±11.53 mg/dI) and with control (154.20±5.49 mg/dI) , and increased no significantly with the third group(227.00±17.82 mg/dI), the second and third group increased significantly comparison with control. There are no significant differences between second and third groups**,** Table (4-2)

4-1-3-2: Triglyceride (TG)

The triglyceride values didn't differ significantly (P<0.05) among first, second and third groups (260.13±25.55, 204.80±10.42 and 261.93±33.86 mg/dI) respectably, while the all three groups increased significantly (P<0.05) when compared to the control (129.47±9.34 mg/dI) (Table 4-2)

4-1-3-3: High density lipoprotein (HDL-C)

The value of (HDL-C) in the first , second and third group(31.07±3.29, 30.47±1.28 and 26.33±1.72 mg/dI) respectably, were decreased significantly (P <0.05) comparison with the control group(45.00±1.50 mg/dI), also the first , second and third group didn't differ significantly (P<0.05) among them, Table (4-2)

4-1-3-4: Low density lipoprotein (LDL-C)

The values of (LDL-C) in the first group (159.53±18.91 mg/dI) increased significantly (P<0.05) in comparison with the second group (114.13±8.44 mg/dI) and the control group (75.07±5.19 mg/dI), but in-

creased not significantly in comparison with the third group (144.53±15.60 mg/dI). Second group (114.13±8.44 mg/dI) increased significantly in comparison with control and third groups, but not differ significantly in comparison with third group. Third group increased significantly in comparison with the control, Table (4-2).

4-1-3-5: Very Low density lipoprotein (VLDL)

The values of VLDL in first (64.33±8.97 mg/dI), second (49.33±5.27 mg/dI) and third groups (54.60±6.80 mg/dI) did not differ significantly (P<0.05) among groups ,while the levels of VLDL was increased significantly (P<0.05) in first, second, and third groups in comparison with the control group (22.53±2.05 mg/dI) (Table , 4-2).

Table (4-2): The values of serum lipid profile in control and patients with DMT2 (According to age)

parameters / Group	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL (mg/dl)
Control(30-59) year	c 154.20±5.49	b 129.47±9.34	a 45.00±1.50	c 75.07±5.19	b 22.53±2.05
First group (30-39)year	a 244.20±15.08	a 260.13±25.55	b 31.07±3.29	a 159.53±18.91	a 64.33±8.97
Second group (40-49)year	b 205.13±11.53	a 204.80±10.42	b 30.47±1.28	b 114.13±8.44	a 49.33±5.25
Third group (50-59)year	ab 227.00±17.82	a 261.93±33.86	b 26.33±1.72	ab 144.53±15.60	a 54.60±6.80
LSD	37.70	63.28	5.97	37.46	17.85

N=15
Values represented mean ± SE.
The different letters refer to significant difference among group at level of (P<0.05).
The same letters refer to non significant difference among group.

4-1-4: Hormonal parameters

4-1-4-1: Follicular stimulating hormone (FSH)

The FSH level in the first (3.03±0.41 mIU/ml), second (2.66±0.24 mIU/ml) and the third groups (3.63±0.34 mIU/ml) were decreased sig-

nificantly (P <0.05) in comparison with the control group (7.03±0.55 mIU/ml). No significant differences in the all three groups . Table (4-3) .

4-1-4-2: Luteinizing hormone (LH)

The levels of the LH in first (2.62±0.36 mIU/ml) , second , (2.20±0.17 mIU/ml) and third , (2.93±0.31 mIU/ml) groups did not differ significantly, however , they decreased significantly (P<0.05) in comparison with the control (4.60±0.36 mIU/ml) group, (Table 4-3).

4-1-4-3: Total Testosterone (TT)

The testosterone (TT) level in the first group (2.60±0.25 ng/ml), the second (2.27±0.17 ng/ml) and the third groups(2.73±0.17 ng/ml) were decreased significantly (P <0.05) in comparison with the control group(4.51±0.23 ng/ml). Also all three groups did not differ significantly (P<0.05) among them, Table (4-3).

4-1-4-4: Estradiol (E2)

The Estradiol (E2) level did not differ significantly (P<0.05) in first (30.61±2.85 pg/ml), second (31.24±1.95 pg/ml) and third groups (25.28±2.11 pg/ml). The first and second groups which increased significantly (P<0.05) in comparison with the control. While there are no significant differences (P<0.05) between control and third group, (Table, 4-3)

4-1-4-5: Prolactin (PRL)

The value of Prolactin (PRL) in the second group (15.89±1.22 ng/ml) increased significantly (P<0.05) in comparison with the third group (10.78±1.52 ng/ml) and with the control group(9.77±0.75 ng/ml), and did not differ significantly (P<0.05) in comparison with the first group (13.83±1.70 ng/ml). First group increased significantly in comparsion

with the control group , but did not differ significantly with third group. Also third group did not differ significantly with control group**,** (Table, 4-3).

Table (4-3): The values of serum hormones parameters in control and patients with DMT2 (According to age)

parameters / Groups	FSH (mlU/ml)	LH (mlU/ml)	TT (ng/ml)	E2 (pg/ml)	PRL (ng/ml)
Control(30-59) year	a 7.03±0.55	a 4.60±0.36	a 4.51±0.23	b 20.28±2.01	b 9.77±0.92
First group (30-39)year	b 3.03±0.41	b 2.62±0.36	b 2.60±0.25	a 30.61±2.85	ab 13.83±1.70
Secondgroup (40-49)year	b 2.66±0.24	b 2.20±0.17	b 2.27±0.17	a 31.24±1.95	a 15.89±1.22
Third group (50-59)year	b 3.63±0.34	b 2.93±0.31	b 2.73±0.17	ab 25.28±2.11	b 10.78±1.52
LSD	1.15	0.89	0.60	6.41	3.92

N=15
Values represented mean ± SE.
The different letters refer to significant difference among group at level of (P<0.05).
The same letters refer non significant difference among groups.

4-2: Biochemical parameters in serum (according to duration)

4-2-1: Fasting Blood Glucose (F.B.G) level

The values of F.B.G in the third group (333.72±19.11 mg/dI) increased significantly (P >0.05) in comparison with the first (266.21±19.46 mg/dI) and control(93.20±2.13 mg/dI) groups. First (266.21±19.46 mg/dI) and second (280.26±34.73 mg/dI) groups increased significantly in comparison with control .No significant difference between third and second groups. No significant difference between second and first groups, Table (4-4).

4-2-2: HbA1C

The HbA1C in the third group (11.20±0.80 %)increased significantly(P >0.05) in comparison with the first (9.37±0.46 %) and the control (5.00±015 %)groups. First (9.37±0.46 %) and second (10.86±0.17 %) group increased significantly in comparison with control group. No significant difference between third and second groups. No significant difference between second and first group groups, Table (4-4).

Table (4-4): The values of F.B.G and HbA1c in control and patient with DM T2 (According to duration of DM)

parameters / Groups	F.B.G (mg/dl)	HbA1C (%)
Control(30-59) year	c 93.20±2.13	c 5.00±0.15
First group (1-5)year	b 266.21±19.46	b 9.37±0.46
Second group (6-10)year	ab 280.26±34.73	ab 10.86±0.37
Third group (11-15)year	a 333.72±19.11	a 11.20±0.80
LSD	59.10	1.52

Values represented mean ± SE.
The different letters refer to the significant difference among group at level of (P<0.05).
The same letters refer to non significant difference among group.

4-2-3: Lipid profile

4-2-3-1: Total Cholesterol (TC)

The cholesterol (TC) values did not differ significantly (P<0.05) among the first (224.69±14.88 mg/dI), second (224.09±12.55 mg/dI) and third (231.54±15.33 mg/dI) groups. But all the groups above (all the three groups) were increased significantly (P<0.05) in comparison with the control (154.20±5.19 mg/dI) group, Table (4-5).

4-2-3-2: Triglyceride (TG)

The values of TG in the third (350.54±57.84 mg/dI) group increased significantly (P<0.05) in comparison with the first(257.13±26.48 mg/dI), second (218.63±19.80 mg/dI) and control(129.46±9.34 mg/dI) group. First and second group increased significantly in comparison with the control. No significant difference between second and first groups, (Table 4-5)

4-2-3-3: High density lipoprotein (HDL-C)

The value of (HDL-C) in the first (30.91±2.41 mg/dI), second(28.90±1.35) and the third (26.54±1.11 mg/dI) group were decreased significantly (P <0.05) in comparison with the control (45.00±1.50 mg/dI)group. As well as the first, second and third groups did not differ significantly among them (P<0.05) , Table (4-5).

4-2-3-4: Low density lipoprotein (LDL-C)

The value of (LDL-C) in the first (141.95±15.33 mg/dI) group did not differ significantly (P <0.05) in comparison with the second (136.63±14.89 mg/dI) and third (122.36±10.83 mg/dI) group. No significant difference between second and first groups. No significant difference between second and third groups. All three groups increased significantly(P<0.05) in comparison with the control group (75.06± 5.19 mg/dI), (Table,4-5).

4-2-3-5: Very Low density lipoprotein (VLDL

The value of VLDL in the third (71.09±10.73) group was increased significantly (P <0.05) in comparison with the first (49.17±5.50 mg/dI) , second (46.54±5.45 mg/dI) and control(22.53±2.05 mg/dI) groups . The first and the second group increased significantly (p<0.05) in comparison

with control group. No significant difference between second and first groups, Table (4-5).

Table (4-5): The values of serum lipid profile in control and patients with DMT2 (According to duration of DM)

parameters / Groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL (mg/dl)
Control(30-59) year	b 156.20±5.49	c 129.46±9.34	a 45.00±1.50	b 75.06±5.19	c 22.53±2.05
First group (1-5)year	a 224.69±14.88	b 257.13±26.48	b 30.91±2.41	a 141.96±15.33	b 49.17±5.50
Second group (6-10)year	a 224.09±12.5	b 218.63±19.8	b 28.90±1.35	a 136.63±14.89	b 46.54±5.45
Third group (11-15)year	a 231.54±15.33	a 350.54±57.89	b 26.54±1.11	a 122.36±10.83	a 71.09±10.73
LSD	39	86.57	6.0	39.34	17.54

Values represented mean ± SE.
The different letters refer to significant difference among group at level of ($P<0.05$).
The same letters refer to non-significant difference among group.

4-2-4: Hormones parameters

4-2-4-1: Follicular stimulating hormone (FSH)

The FSH values in the first(2.92±0.26 mUI/mI) , second(2.97±0.46 mUI/mI) and the third(3.63±0.41 mUI/mI) group were decreased significantly ($P <0.05$) in comparison with the control(7.03±0.55 mUI/mI) group. While no significant difference were recorded in the level of FSH among first, second and third groups, Table (4-6).

4-2-4-2: Luteinizing hormone (LH)

The LH values in the first (2.29±0.20 mUI/mI), second (2.88±0.33 mUI/mI)and third (3.05±0.39 mUI/mI)groups were decreased signifi-

cantly (P<0.05) in comparison with the control(4.60±0.36 mUI/mI) group. While no significant difference were recorded in the level of LH among first , second and third groups, Table (4-6).

4-2-4-3: Total Testosterone (TT)

The testosterone (TT) values in the first (2.50±0.14 ng/mI), second(2.33±0.28 ng/mI) and the third(2.68±0.23 ng/mI) group were decreased significantly (P<0.05) in comparison with the control(4.51±0.23 ng/mI) group. While no significant difference were recorded in the level of (TT) among first, second and third groups, Table (4-6)

4-2-4-4: Estradiol (E2)

The Estradiol (E2) values which increased significantly (P<0.05) in the first (28.25±1.88 pg/mI), second (28.05±2.97 pg/mI) and third (28.66±3.04 pg/mI) groups in comparison with the control (20.28±2.01 pg/mI). While no significant difference were recorded in the level of (E2) among first, second and third groups, Table (4-6).

4-2-3-5: Prolactin (PRL)

The Prolactin (PRL) values were increased significantly (P<0.05) in the first (15.37±1.27 ng/mI), second (13.12±1.45 ng/mI) and third (13.19±1.63 ng/mI) groups in comparison with the control (9.77±0.95 ng/mI). While no significant difference were recorded in the level of prolactin among first , second and third groups, Table (4-6).

Table (4-6): The values of serum hormones parameters in control and patients with DMT2 (According to duration of DMT2)

parameters / Groups	FSH (mlU/ml)	LH (mlU/ml)	TT (ng/ml)	E2 (pg/ml)	PRL (ng/ml)
Control(30-59) year	a 7.03±0.55	a 4.60±0.36	a 4.51±0.23	b 20.28±2.01	b 9.77±0.95
First group (1-5)year	b 2.90±0.26	b 2.29±0.20	b 2.50±0.14	a 28.25±1.88	a 15.37±1.27
Secondgroup (6-10)year	b 2.97±0.46	b 2.88±0.33	b 2.33±0.28	a 28.05±2.97	a 13.12±1.45
Third group (11-15)year	b 3.63±0.41	b 3.03±0.59	b 2.68±0.23	a 28.66±3.04	a 13.19±1.63
LSD	1.16	0.86	0.61	6.65	3.83

Values represented mean ± SE.
The different letters refer to significant difference among group at level of (P<0.05).
The same letters refer to non significant difference among group.

4-3: Semen parameters (According to age)

4-3-1: Macroscopic parameters

4-3-1-1: pH

The pH in the second group (7.80±0.60) was decreased significantly (P <0.05) in comparison with the control group(8.00±0.00) control group did not differ significantly with the first group(7.84±0.08), while the first group did not differ significantly (P<0.05) in comparison with the second group (Figure 4-1,Table4-7).

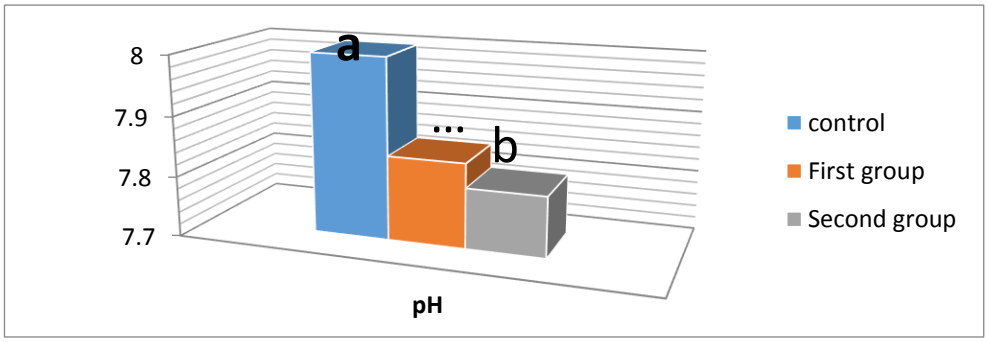


Figure (4-1): The semen pH values for different group in diabetic men and control

4-3-1-2: Volume

No significant differences (P<0.05) in the semen volume in all groups (Figure 4-2, Table 4-7).

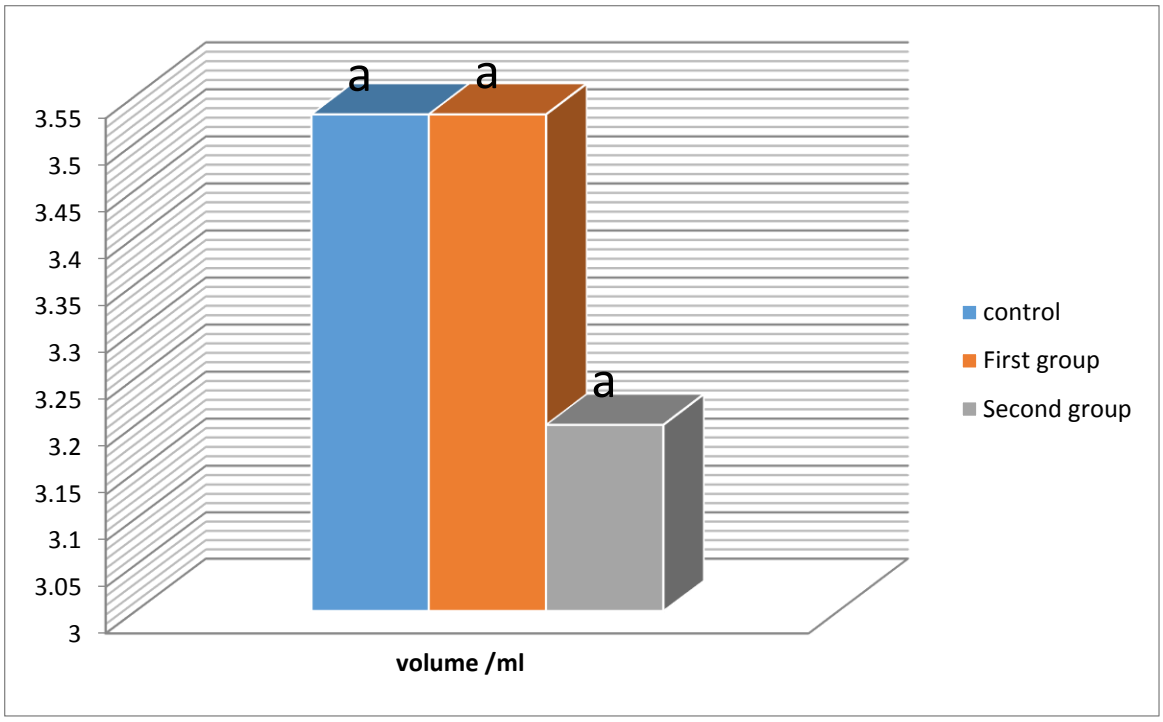


Figure (4-2):The volume values of semen in T2DM and control men.

4-3-1-3: Liquefaction

The Liquefaction in the first group(34.00±2.72 min) was significantly increased (P <0.05) in comparison with the control group(29.33±0.66 min), and did not differ significantly with the second group(30.00±0.00 min), while second group did not differ significantly (P<0.05) in comparison with the control group (Figure 4-3,Table 4-7,).

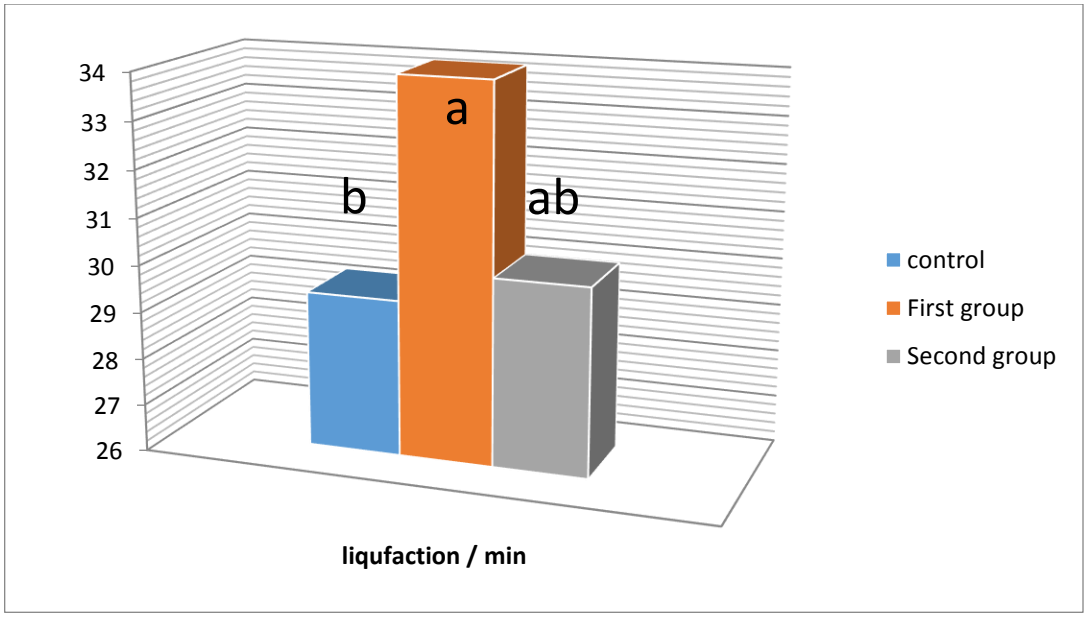
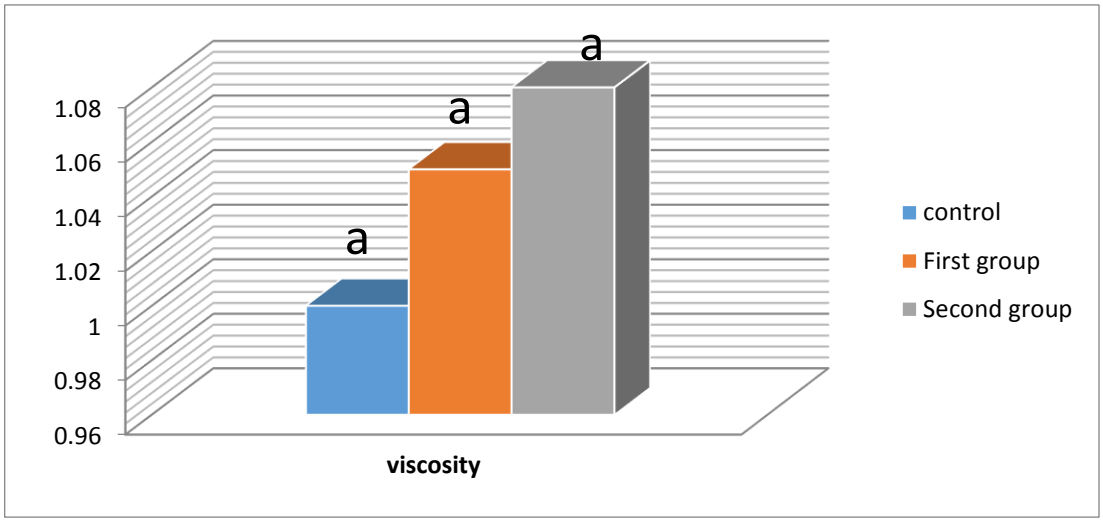


Figure (4-3): The liquefaction time of semen in T2DM and control men

4-3-1-4: Viscosity

There are no significant differences (P<0.05) in the viscosity in control (1.00±0.03), first(1.05±0.05) and second(1.08±0.09) groups (Figure4-4,Table 4-7).



Figure(4-4):The viscosity values of semen in T2DM and control men

4-3-2: Microscopic parameters

4-3-2-1: Concentration

The concentration of sperm in first (27.66±5.72 10^6/ml) and second (37.66±3.41 10^6/ml) group were decreased significantly (P <0.05) in comparison with the control group (62.46±4.20 10^6/ml)group, but first and second groups not differ significantly (P<0.05) between them (Figure 4-5,Table 4-7).

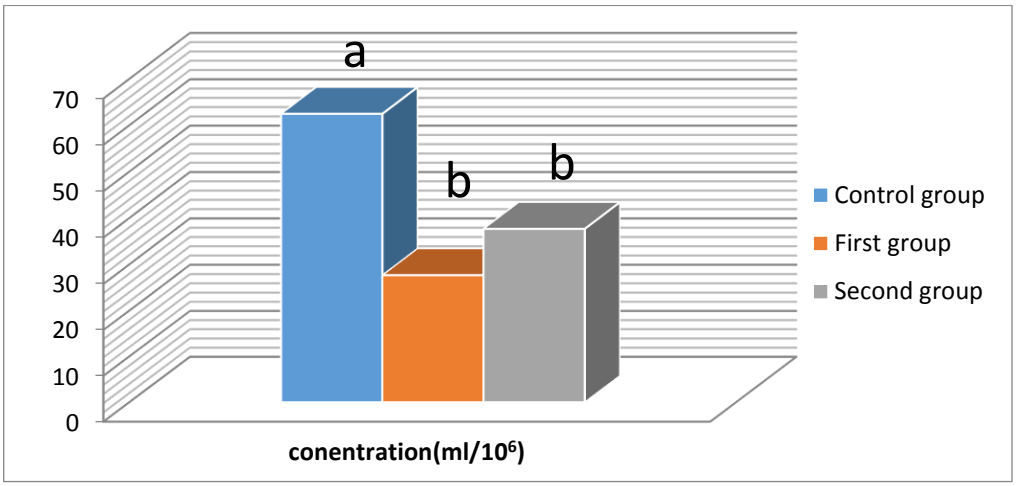


Figure (4-5): The concentration of sperm in T2DM and control men

4-3-2-2: Motility

4-3-2-2-1: progressive (A)

The motility (A) of sperm in first (5.33±1.72 %) and second (8.00±1.52 %) group were decreased significantly (P <0.05) in comparison with the control (31.33±2.46 %) group, but first and second groups not differ significantly (P<0.05) between them (Figure 4-6, Table 4-7).

4-3-2-2-2: Non-progressive(B)

The motility (B) of sperm in first (14.33±2.42 %) and second(16.33±1.96 %) group were decreased significantly (P <0.05) in comparison with the control (34.66±1.24 %) group, but first and sec-

ond groups not differ significantly (P<0.05) between them (Figure 4-6, Table 4-7).

4-3-2-2-3: Sluggish (C)

The motility (C) of sperm in the second (30.66±2.84 %) group was increased significantly (P- <0.05) in comparison with the control (15.00±1.54 %) but not differ significantly with the first (30.33±4.21 %) group, while all groups increased significantly (P<0.05) in comparison with the control group (Figure 4-6,Table 4-7).

4-3-2-2-4: Dead (D)

The motility (D) of sperm in the first (50.00±8.35 %) and second (45.00±4.90 %) groups were increased significantly (P<0.05) in comparison with the control (19.00±1.90 %) group, but first group did not differ significantly (P<0.05) in comparison with the second group (Figure 4-6, Table 4-7).

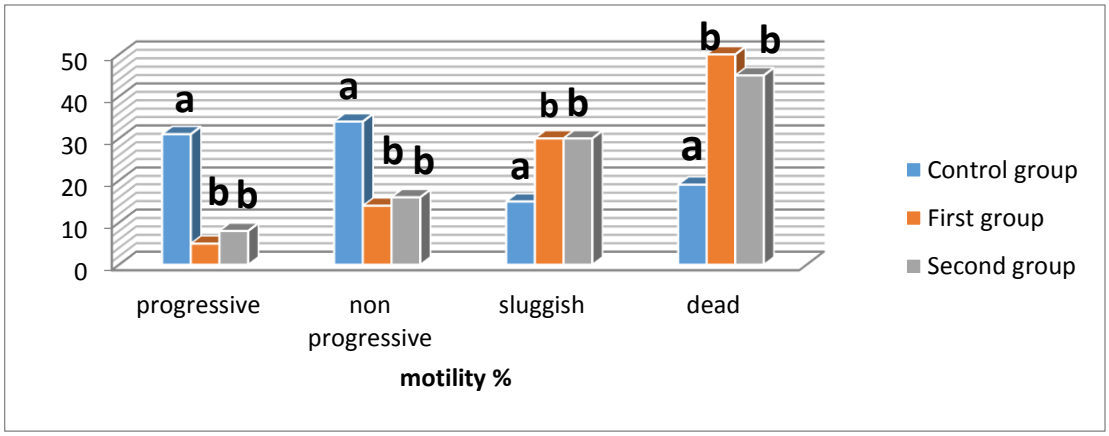


Figure (4-6): The percentage of sperm motility in T2DM and control men

4-3-2-3: Morphology

4-3-2-3-1: Normal

Normal morphology of sperm in first (46.00±7.71 %) and second (59.33±3.34 %) groups were decreased significantly (P <0.05) in com-

parison with the control (82. ±1.52 %) group, but second group did not differ significantly (P<0.05) in comparison with the first group (Figure 4-7, Table 4-7,).

4-3-2-3-2: Abnormal

Abnormal morphology of sperm in the first (54.00±6.51 %) and second (40.66±3.34 %) groups was increased significantly (P <0.05) in comparison with the control (18.00±1.52 %) group, also first group differ significantly (P<0.05) in comparison with the second group (Figure 4-7, Table 4-7).

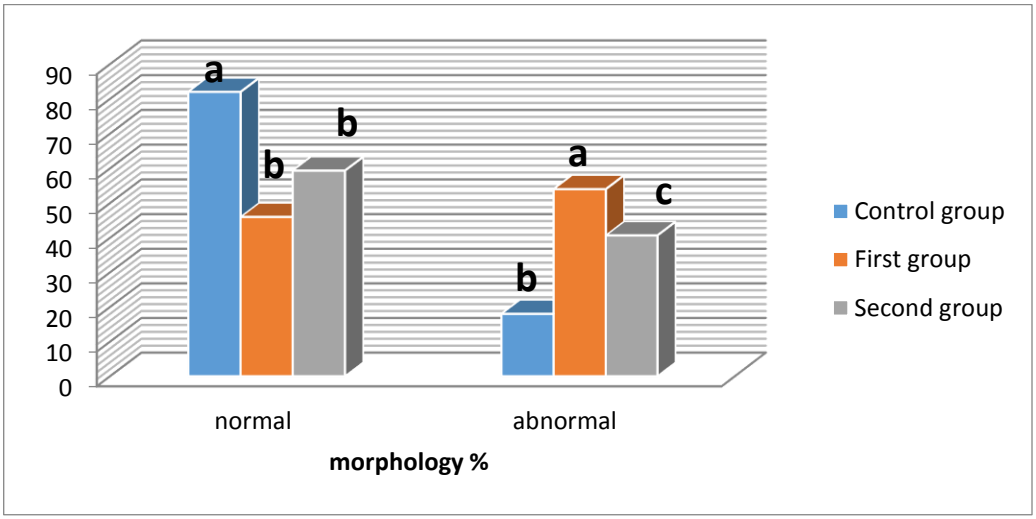


Figure (4-7): The percentage of sperm morphology in T2DM and control men

4-4: Biochemical parameters of seminal plasma

4-4-1: Fructose

No significant variation was recorded in the value of fructose in the first and second groups (8.11±1.86, 9.34±1.60 mg/ml) respectively in comparison with the control (5.51±0.94 mg/ml) (Figure 4-8, Table 4-7).

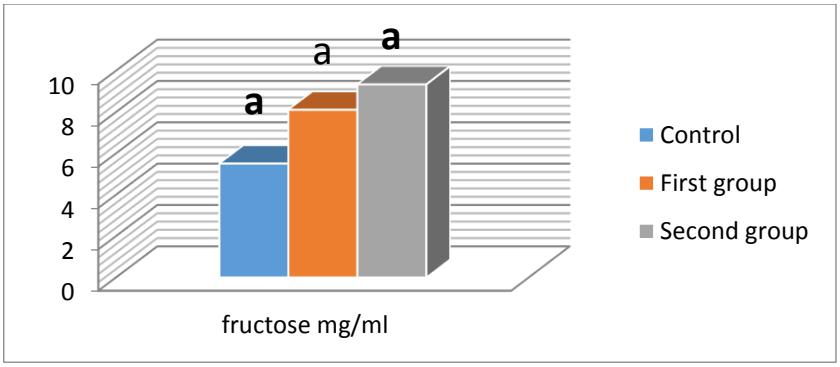


Figure (4-8): The fructose values of seminal plasma in T2DM and control men.

4-4-2: Zinc (Zn)

The zinc values in first (141.86±5.38 µg/mI) and second (132.00±3.47 µg/mI) groups were decreased significantly (P<0.05) in comparison with the control (162.93±9.18 µg/mI) group, while first and second group did not differ significantly (P<0.05) between them (Figure 4-9,Table 4-7).

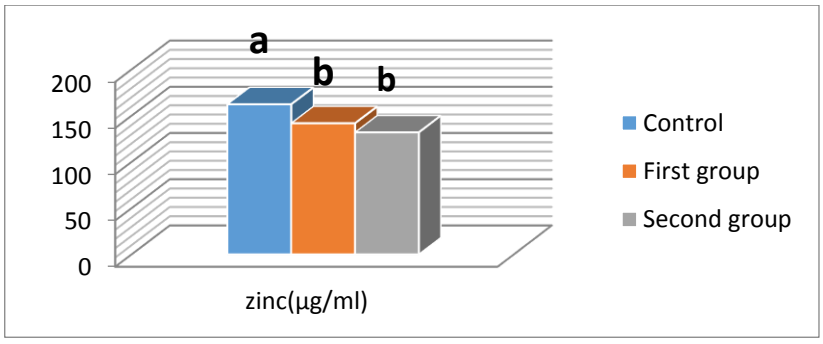
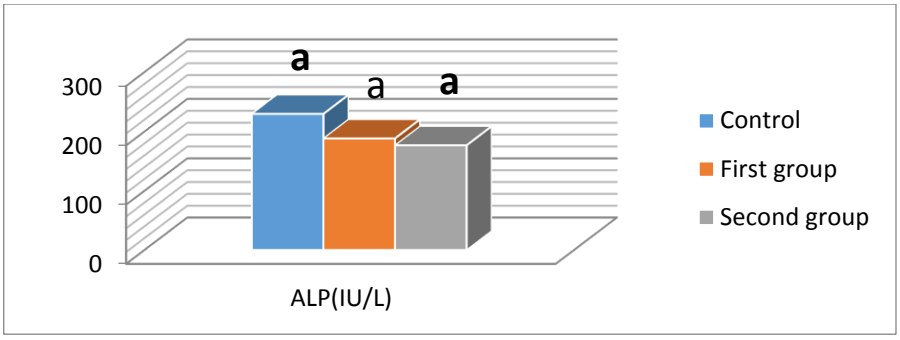


Figure (4-9): The zinc values of seminal plasma inT2DM and control men.

4-4-3: Alkaline phosphatase (ALP)

No significant variation was recorded in the value of ALP in the first and second groups (188.48±17.77, 176.91±25.42 IU/L)respectively in comparison with the control(229.70±24.82 IU/L) (Figure 4-10, Table 4-7).



Figure(4-10): The ALP values of seminal plasma in T2DM and control men .

4-4-3: Glutathione (GSH)

The GSH values in first (13.88±0.92 ng/L) and second (13.87±0.64 ng/L) groups were decreased significantly (P<0.05) in comparison with the control (17.64±0.82 ng/L)group, but first and second group did not differ significantly (P<0.05) between them(Figure 4-11, Table 4-7).

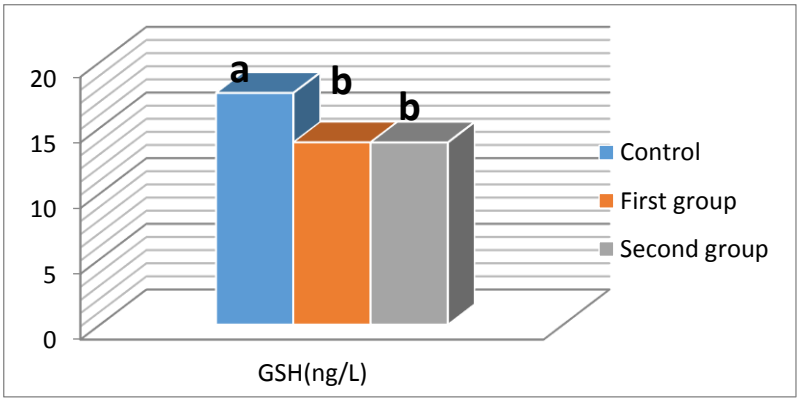


Figure (4-11): The GSH values of seminal plasma in T2DM and control men.

4-5: Semen parameters (According to duration of T2DM)

4-5-1: Macroscopic parameters

4-5-1-1: pH

The pH in the first and second groups (7.76±0.07, 7.90±0.60) respectively decrease significantly (P<0.05) in comparison with the con-

trol (8.00±0.00) group, while the first and second groups did not differ significantly between them (P<0.05) (Figure 4-12, Table4-8).

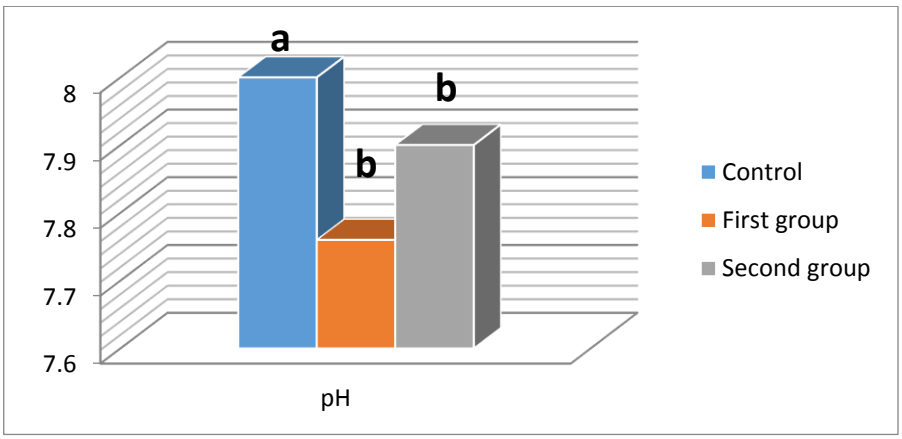
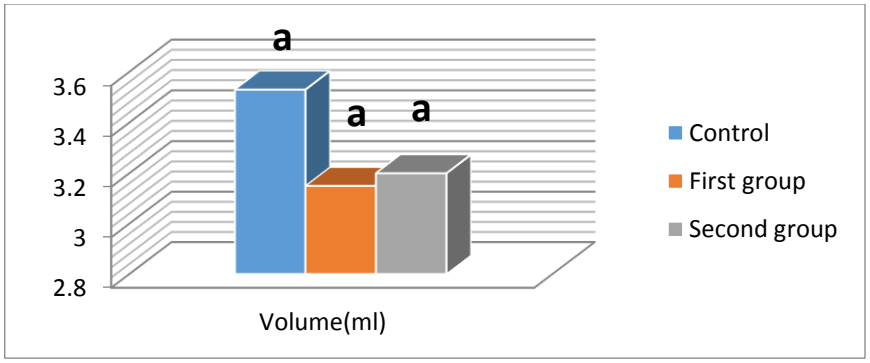


Figure (4-12): The semen pH values for different group in diabetic men and control.

4-5-1-2: Volume

The volume no significant differences (P<0.05) in the semen volume in all groups (Figure 4-13, Table 4-8).



Figure(4-13): The volume values of semen in T2DM and control men.

4-5-1-3: Liquefaction

The Liquefaction in the first (31.57±1.57 min), second(32.72±2.72 min) and control (29.33±0.66 min) groups did not show significant differences(P<0.05) (Figure 4-14, Table 4-8).

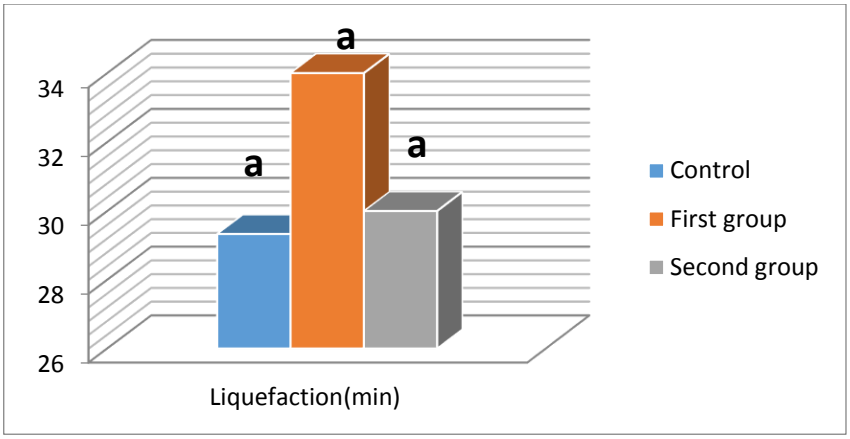


Figure (4-14): The liquefaction time of semen in T2DM and control men.

4-5-1-4: Viscosity

The viscosity in the first (1.05±0.05), second (1.09±0.09) and control(1.00±0.03) groups did not show significant difference (P<0.05)(Figure 4-15, Table 4-8).

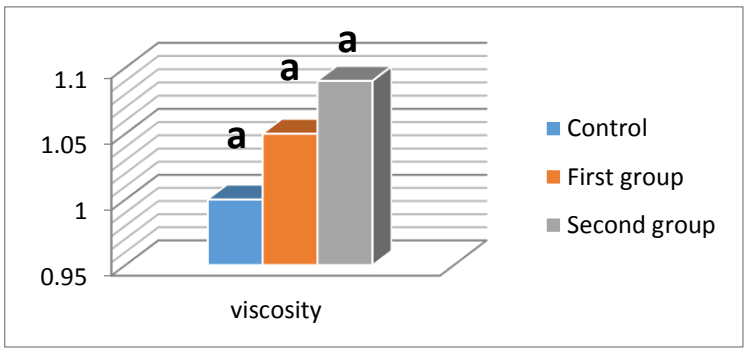


Figure (4-15):The viscosity values of semen in T2DM and control men.

4-5-2 Microscopic parameters

4-5-2-1 Concentration

The concentration of sperm in first (35.05±4.53 10^6/ml) and second(28.54±4.98 10^6/ml) groups were decreased significantly (P<0.05) in comparison with the control(62.46±4.20 10^6/ml) group, but first and second groups not differ significantly (P<0.05) between them (Figure 4-16, Table 4-8).

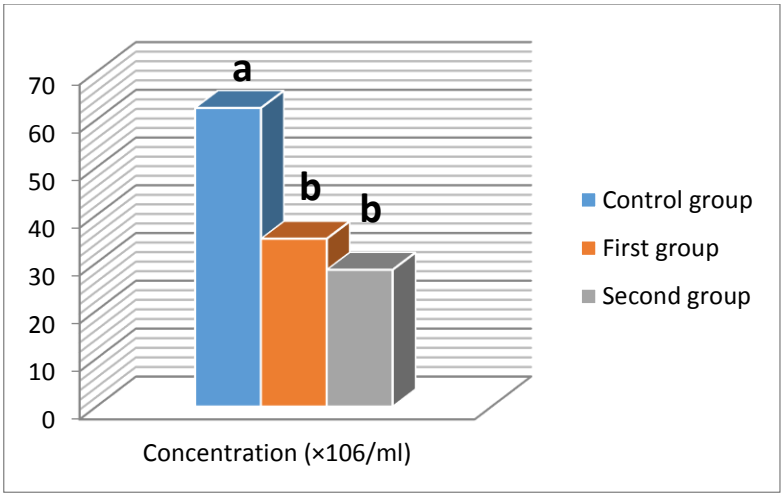


Figure (4-16): The concentration of sperm in T2DM and control men.

4-5-2-2: Motility

4-5-2-2-1: progressive (A)

The motility (A) of sperm in first (6.97±1.74 %) and second (7.72±1.23 %) group were decreased significantly (P <0.05) in comparison with the control(31.33±2.46 %) group, but first and second groups not differ significantly (P<0.05) between them (Figure 4-17, Table 4-8).

4-5-2-2-2: Non-progressive(B)

The motility (B) of sperm in first (15.39±1.81 %) and second (17.74±1.23 %) group were decreased significantly (P <0.05) in comparison with the control(34.66±1.24 %) group, but first and second groups not differ significantly (P<0.05) between them (Figure 4-17, Table 4-8).

4-5-2-2-3: Sluggish (C)

The motility (C) in the second (35.90±2.76 %) group was increased significantly (P <0.05) in comparison with the first (22.63±2.39 %) and the control (15.00±1.54 %)groups, also first and second groups differences significantly (P<0.05) between them. (Figure 4-17, Table 4-8).

4-5-2-2-4: Dead (D)

The motility (D) of sperm in first (55.00±4.56 %) and second (38.63±2.86 %) group were increased significantly (P <0.05) in comparison with the control(19.00±1.90 %) group, also first and second groups differ significantly (P<0.05) between them (Figure 4-17, Table 4-8).

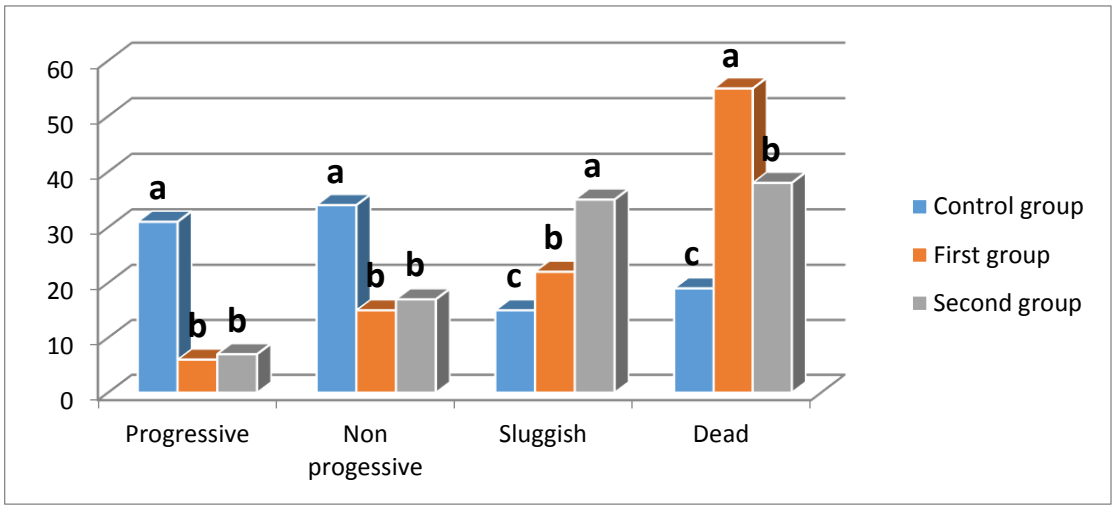


Figure (4-17): The percentage of sperm motility in T2DM and control men.

4-5-2-3: Morphology

4-5-2-3-1: Normal

Normal morphology of sperm in first (60.26±3.94 %) and second (51.81±3.83 %) groups were decreased significantly (P <0.05) in comparison with the control(82. ±1.52 %) group, but first group did not differ significantly (P<0.05) in comparison with the control group (Figure 4-18, Table 4-8).

4-5-2-3-2: Abnormal

Abnormal morphology of sperm in the first (39.73±3.93 %) and second(48±3.83 %) groups was increased significantly (P <0.05) in com-

parison with the control (18.00±1.52 %)group, but first group did not differ significantly(P<0.05) compared to the second group (Figure 4-18, Table 4-8,).

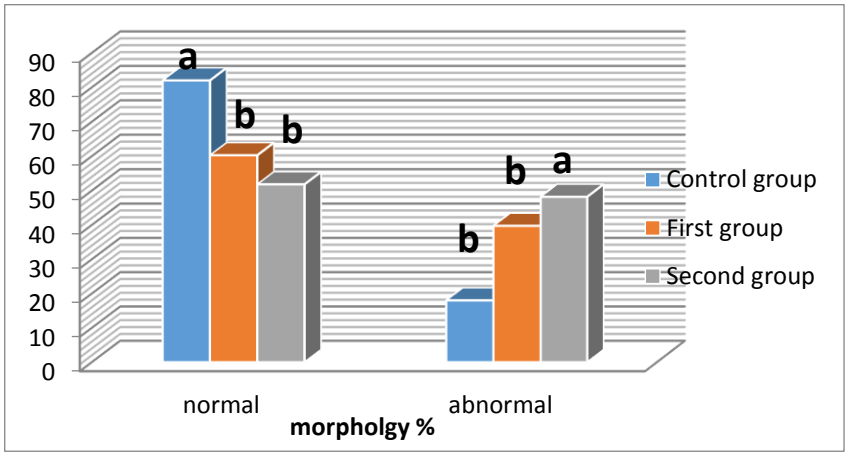


Figure (4-18): The percentage of sperm morphology in T2DM and control men.

4-6: Biochemical parameters of seminal plasma

4-6-1: Fructose

No significant variation was recorded in the value of fructose in the first and second groups (8.166±1.63, 9.56±1.81 mg/ml) respectively in comparison with the control (5.51±0.94 mg/ml) (Figure 4-19, Table 4-8).

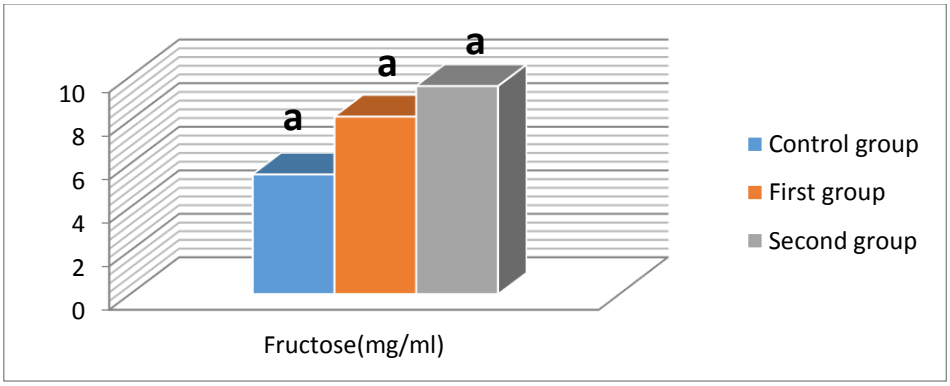
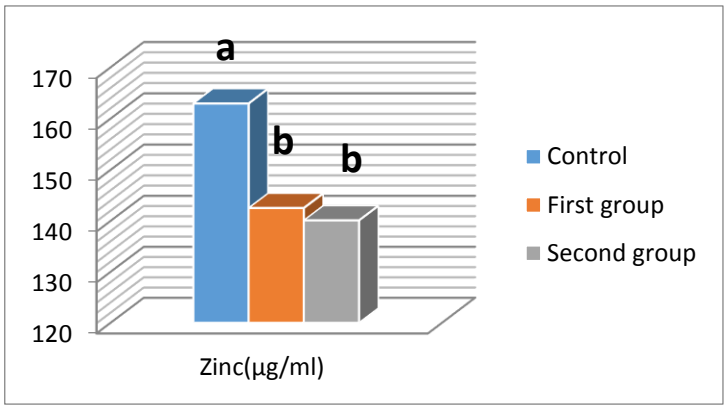


Figure (4-19): The fructose values of seminal plasma in T2DM and control men.

4-6-2: Zinc (Zn)

The zinc values in first (142.52±5.38 µg/mI) and second(140.09±5.49 µg/mI) groups were decreased significantly (P<0.05) in comparison with the control (162.93±9.18 µg/mI)group, but first and second group did not differ significantly (P<0.05) between them (Figure 4-20,Table 4-8).



Figure(4-20):The Zinc values of seminal plasma in and control men.

4-6-3: Alkaline phosphatase (ALP)

No significant variation was recorded in the value of ALP in the first and second groups (186.75±18.08, 226.60±25.90 IU/L) respectively in comparison with the control (229.70±24.82 IU/L) (Figure 4-21, Table 4-8)

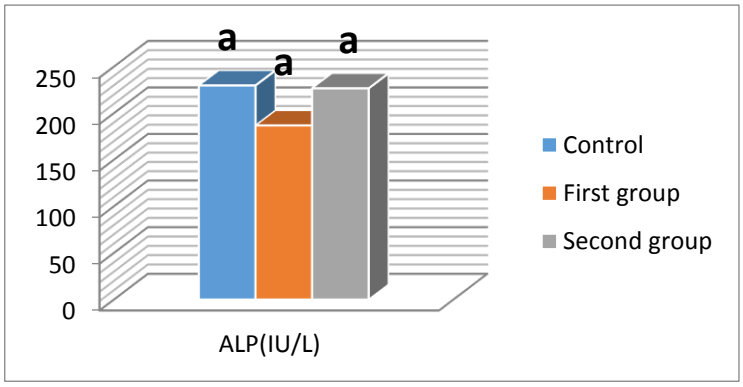


Figure (4-21): The ALP values of seminal plasma in T2DM and control men.

4-6-4: Glutathione (GSH)

The GSH values in first (14.47±0.72 ng/L) and second (15.81±0.55 ng/L) groups were decreased significantly (P<0.05) in comparison with the control (17.64±0.82 ng/L)group, also first and second group did not differ significantly (P<0.05) between them (Figure 4-22, Table 4-12)

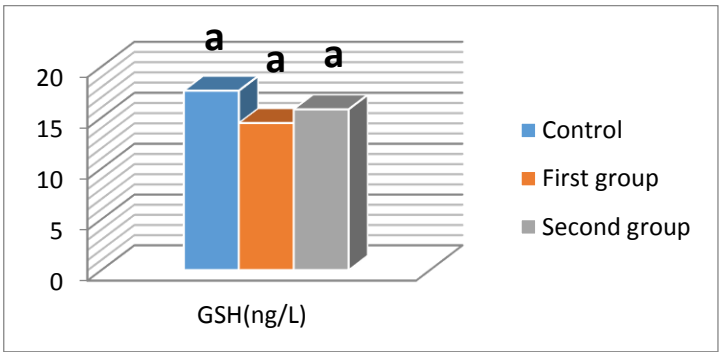


Figure (4-22): The GSH values of seminal plasma in T2DM and control men.

Table(4-7):parameter of semen in diabetic mellitus type 2 and control (According to age)

Macroscopic examination of semen					
Parameters \ **Groups**		**Control (30-59)year**	**First group (30-39)year**	**Second group (40-49)year**	LSD
PH		a 8.00±0.00	a b 7.84±0.08	b 7.80±0.60	0.17
Volume (ml)		3.53±0.21	3.13±0.37	3.20±0.38	NS
Liquefaction (min)		b 29.33±0.66	a 34.00±2.72	ab 30.00±0.66	4.62
Viscosity		1.00±0.03	1.05±0.05	1.08±0.09	NS
Microscopic examination of semen					
Concentration ($\times 10^6$/ml)		a 62.46±4.20	b 27.66±5.72	b 37.66±3.41	12.96
Motility %	Progressive(A)	a 31.33±2.46	b 5.33±1.72	b 8.00±1.52	5.54
	Non- progres-sive(B)	a 34.66±1.24	b 14.33±2.42	b 16.33±1.96	5.53
	Sluggish (C)	b 15.00±1.54	a 30.33±4.21	a 30.66±2.84	8.73
	Dead (D)	b 19.00±1.90	a 50.00±8.35	a 45.00±4.90	16.24
Morphology %	Normal	a 82.00±1.52	b 46.00±7.71	b 59.33±3.34	14.06
	Abnormal	b 18.00±1.52	a 54.00±6.51	c 40.66±3.34	12.30
Biochemical parameters of semen					
Fructose(mg/ml)		5.51±0.94	8.11±1.86	9.34±1.60	NS
Zinc(µg/ml)		a 162.93±9.18	b 141.86±5.38	b 132.00±3.47	18.42
ALP(IU/L)		229.70±24.82	188.48±17.77	176.91±25.42	NS
GSH(ng/L)		a 17.64±0.82	b 13.88±0.92	b 13.87±0.64	2.29

N= 15

Values represented mean ± SE, Same letters refer to no significant (P<0.05)
The different letters refer to significant difference among group at level of (P<0.05).
NS represented non significant among group.

Table (4-8): parameters of semen in diabetic type 2 and control (According to duration)

Macroscopic examination of semen					
Parameters \ Groups		Control (30-59)	First group (1-5) year	Second group (6-10)year	LSD
PH		a 8.00±0.00	b 7.76±0.07	ab 7.90±0.60	0.16
Volume (ml)		3.53±0.21	3.15±0.32	3.20±0.38	NS
Liquefaction (min		29.33±0.66	34.00±2.72	30.00±0.66	NS
Viscosity		1.00±0.03	1.05±0.05	1.09±0.09	NS
Microscopic examination of semen					
Concentration ($\times 10^6$/ml)		a 62.46±4.20	b 35.05±4.53	b 28.54±4.98	13.18
Motility %	Progressive(A)	a 31.33±2.46	b 6.97±1.72	b 7.72±1.23	5.26
	Non- progressive(B)	a 34.66±1.24	b 15.39±1.81	b 17.74±1.23	4.56
	Sluggish (C)	c 15.00±1.54	b 22.63±2.39	a 35.90±2.76	6.53
	Dead (D)	c 19.00±2.90	a 55.00±4.35	b 38.63±2.86	10.26
Morphology %	Normal	a 82.00±1.52	b 60.26±3.94	b 51.81±3.83	9.78
	Abnormal	b 18.00±1.52	a 39.73±3.94	a 48.18±3.83	9.78
Biochemical parameter of semen					
Fructose(mg/ml)		5.51±0.94	8.16±1.63	9.56±1.81	NS
Zinc(µg/ml)		a 162.93±9.18	b 142.52±5.38	b 140.09±5.49	19.97
ALP(IU/L)		229.70±24.82	186.75±18.08	226.60±25.90	NS
GSH(ng/L)		a 17.64±0.82	b 14.47±0.72	b 15.81±0.55	2.11

Values represented mean ± SE, Same letters refer to no significant($P<0.05$)
The different letters refer to significant difference among group at level of ($P<0.05$).
NS represented non significant among group.

Summary

Summary

The aim of the present study was to determine the effect of type 2 diabetes on the levels of lipids profile , reproductive hormones in serum and fertility measurements in semen of the sample of men at Misan Province for the period from February 2018 to November 2018 . The study included 60 men (45 diabetic and 15 healthy as control group), the average age between 30 to 59 years. The number of patients divided according to age into three groups, the first (30-39) years , second (40-49) years and third (50-59), also divided by the duration of diabetes to three groups the first (1-5) year , second (6-10) year and third (11-15) years, when measuring lipid levels and reproductive hormones. But fertility measurement included the first and second groups only for both age and duration of diabetes the results showed that :

The result of the present study showed a significant inecrease ($P<0.05$) in fasting blood glucose and HbA1c diabetes patients groups compare with control group for age and duration of diabetes. Also there was a significant increase in cholesterol, TG, LDL and VLDL in diabetes group compared with control, while there was a significant decrease in HDL of the diabetes groups compared with control group for age and duration diabetes. The result decreased a significant in FSH, LH and TT in diabetes groups compared with control, while ther was a significant increased in PRL and E2 in diabetes groups compared with control group for age and duration diabetes.

The pH of semen in the second group age (40-49 year) group significantly decreased ($P <0.05$) in diabetes compared with control group , while the first age (30-39 year) group did not differ significantly ($P <0.05$) compared with control group, but the pH in the first (1-5 year)

group decreased significantly (P<0.05) in diabetic group compared with control according to the duration. Volume and viscosity of semen did not differ significantly (P <0.05) groups compared to control according to age and duration of diabetes. Liquefaction in first age (30-39 year) group increased significantly (P <0.05) compared to control, while the second age (40-49 year) group did not differ significantly (P <0.05) compared to control, while liquefaction did not differ significantly (P <0.05) compared to control according to duration of diabetes.

The result of the present study showed a significant decreased in concentration of sperm , progressive motility, non progressive and normal morphology decreased in all group according to age and duration compare with control , while the sluggish and dead and abnormal morphology increased significantly (P <0.05) in the diabetes groups compared to control group according to each of age and duration.

The values of fructose and alkaline phosphatase in the seminal plasma at the first group (30-39 years) and second group (40-49 years) were not differ significantly (P <0.05) compared with control both in age and duration of diabetes . Zinc and glutathione concentration decreased significantly (P <0.05) in comparison with control in each of age and duration of diabetes.

Conclusion: Dyslipidemia in patients with type 2 diabetes . Decrement of gonadotropin hormones (FSH,LH) and TT and increased of PRL and E2 may be effected on male fertility.

الخلاصة

هدفت الدراسه الحاليه الى بيان تاثير مرض السكري من النوع الثاني على مستويات الدهون والهرمونات التكاثريه في المصل وقياسات الخصوبه في السائل المنوي لدى عينة من الرجال في محافظة ميسان للفتره من شباط 2018 لغايه تشرين الثاني 2018. شملت الدراسه 60 رجلا (45 مصاب بالسكري و 15 سليما كمجموعة سيطره)، تراوحت اعمارهم بين 30-59 سنة. قسم المصابين حسب العمر الى ثلاث مجاميع ، الاولى (30- 39) سنه والثانيه (40-49)سنه والثالثه (50- 59) ، بينما قسم حسب مدة الاصابه بمرض السكري الى ثلاث مجاميع ايضا الاولى (1- 5) سنه والثانيه (6- 10) سنه والثالثه (11-15) سنه ، عند قياس مستويات الدهون والهرمونات التكاثريه. بينما شملت قياسات الخصوبه المجاميع العمريه الاولئ والثانيه فضلا عن مدة الاصابه الاولى والثانيه بينت النتائج ما يلي :

أظهرت نتائج الدراسة انخفاضا معنويا عن مستوى احتماليه ($P<0.05$) في مستوى سكر الدم FBG والسكر التراكمي HbA1C في المجاميع العمريه ومدة الاصابه لمرضى السكري مقارنة مع مجموعة السيطره ، كذلك بينت ارتفاعا معنويا في مستوى الكوليسترول ، الدون واطئة الكثافه LDL والدهون واطئة الكثافه جدا VLDL في مجاميع مرضى السكري مقارنة مع مجموعه السيطرة حسب العمر ومدة الاصابه ، ايضا اظهرت انخفاضا معنويا في الهورمون المحرض للجريبات FSH وللوتيني LH والتيستترون الكلي في مجاميع مرضى السكري مقارنة مع مجموعة السيطره ، بينما كان هناك ارتفاعا معنويا في مستوى البرولاكتين والاستراديول في مجاميع مضى السكري مقارنة مع مجموعه السيطره حسب العمر ومدة الاصابه بالمرض.

أنخفض الرقم الهيدروجيني للسائل المنوي في المجموعة العمريه الثانية (40-49 سنة) بشكل معنوي ($P <0.05$) في مجموعة مرض السكري مقارنة مع مجموعة السيطرة ، في حين أن المجموعة العمرية الأولى (30-39 سنة) لم تختلف معنويا ($P <0.05$) مقارنة بـ السيطرة بيمنا اظهرت مدة الاصابة المجموعه الأولى(1-5 سنة) انخفاضا معنويا ($P <0.05$) في قيمة الرقم الهيدروجيني . لم يختلف الحجم واللزوجة اختلافًا معنويا ($P <0.05$) في مجاميع مرضى السكري مقارنةً بالسيطره حسب العمر ومدة الاصابة بالمرض. ازدادت الاماعه في المجموعة العمرية الأولى (30-39 سنة) بشكل معنوي ($P <0.05$) مقارنة بالسطره ، بينما لم تختلف المجموعة العمريه الثانية (40-49 سنة)

معنويا (P <0.05) مقارنة بالسيطره وفقًا للعمر ، في حين لم تختلف الاماعه اختلافًا معنويا (P <0.05) مقارنة بالسيطره حسب مدة الاصابه .

أنخفض التركيزالحيامن ونسب الحركة التدريجيه ،غير التدريجيه والشكل الطبيعي بشكل معنوي (P <0.05) ، في حين أن نسب الحركة البطيئة، الميتة والشكل غير الطبيعي زادت بشكل معنوي (P <0.05) في مجموعتي مرضى السكري مقارنة بمجموعة السيطره وفقا للعمر ومدة الاصابه بالمرض.

لم تختلف قيم الفركتوز وانزيم الفوسفاتيز القلوي في بلازما السائل المنوي للمجموعه الاولى الأولى (30-39 سنة) والثانيه (40-49 سنة) اختلافا معنويا(P <0.05) عند مرضى السكري مقارنة بمجوعة السيطره حسب العمر ومدة الاصابه بالمرض . انخفض الزنك والغلوتاثيون انخفاضا معنويا (P <0.05) عند مرضى السكري مقارنه بمحموعة السيطره حسب العمر ومدة الاصابه بالمرض.

الاستناجات :ارتفاع الدهون في مرضى السكري من النوع الثاني ، انخفاض هرمونات التناسلية ربما يكون لها تاثير على خصوبة الرجال .

Appendixes

Appendix (1)

Questionnaire

<table>
<tr><td colspan="3">Case No.</td><td colspan="2"></td></tr>
<tr><td colspan="3">Name</td><td colspan="2"></td></tr>
<tr><td colspan="3">Age</td><td colspan="2"></td></tr>
<tr><td colspan="3">Social status</td><td colspan="2"></td></tr>
<tr><td colspan="3">Number of year marriage</td><td colspan="2"></td></tr>
<tr><td colspan="3">Number of children</td><td colspan="2"></td></tr>
<tr><td colspan="3">Duration of diabetes</td><td colspan="2"></td></tr>
<tr><td colspan="3">Are you suffer from(cardiac disease, hyperten-sion, acute and chronic infections, renal disease, hepatic dysfunction, , prostate surgery and dysthyroidism</td><td colspan="2"></td></tr>
<tr><td colspan="3">[Does he have problem in fertility?</td><td colspan="2"></td></tr>
<tr><td colspan="3">Blood examination</td><td colspan="2" rowspan="14">Blood analysis</td></tr>
<tr><td colspan="2">HbA1C</td><td></td></tr>
<tr><td colspan="3">Serum examination</td></tr>
<tr><td colspan="2">F.B.G</td><td></td></tr>
<tr><td colspan="2">TC</td><td></td></tr>
<tr><td colspan="2">TG</td><td></td></tr>
<tr><td colspan="2">HDL-C</td><td></td></tr>
<tr><td colspan="2">LDL-C</td><td></td></tr>
<tr><td colspan="2">VLDL</td><td></td></tr>
<tr><td colspan="2">FSH</td><td></td></tr>
<tr><td colspan="2">LH</td><td></td></tr>
<tr><td colspan="2">TT</td><td></td></tr>
<tr><td colspan="2">PRL</td><td></td></tr>
<tr><td colspan="2">E2</td><td></td></tr>
<tr><td colspan="3">Macroscopic examination of semen</td><td colspan="2">Biochemical parameters of seminal plasma</td></tr>
<tr><td colspan="2">pH</td><td></td><td>Fructose</td><td></td></tr>
<tr><td colspan="2">Volume</td><td></td><td>Zinc</td><td></td></tr>
<tr><td colspan="2">Liquefaction</td><td></td><td>Alkaline phosphatase</td><td></td></tr>
<tr><td colspan="2">Viscosity</td><td></td><td>Glutathione</td><td></td></tr>
<tr><td colspan="3">Microscopic examination of semen</td><td colspan="2" rowspan="7">Seminal fluid analysis</td></tr>
<tr><td colspan="2">Concentration</td><td></td></tr>
<tr><td rowspan="4">Motility</td><td>Progressive</td><td></td></tr>
<tr><td>Non pro-gressive</td><td></td></tr>
<tr><td>Sluggish</td><td></td></tr>
<tr><td>Dead</td><td></td></tr>
<tr><td rowspan="2">Morphology</td><td>Normal</td><td></td></tr>
<tr><td>Abnormal</td><td></td></tr>
</table>

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