Ministry of Higher Education &

Scientific Research

University of Diyala / College of Science

Department of Biotechnology

Morning study

Detection of the presence of microglubulin B2 genes by PCR technique in women suffering from reproductive disorders in Diyala prevalence

Graduate thesis submitted to Department of Biotechnology

Council / College of Science / University of Diyala

It is part of the requirements for obtaining a bachelor's degree in Biotechnology

By

Nabaa Adeeb

Safa Adnan

Nargis Amer

Supervised by

Dr. Alyaa Maan Andelhameed

2020_2021

.

خَلَقَ الإِنسَانَ مِنْ عَلَقٍ*

اقْرَأْ وَرَبُّكَ الأَكْرَمُ*

الَّذِي عَلَّمَ بِالْقَلَمِ *

عَلَّمَ الإِنسَانَ مَا لَمْ يَعْلَمْ*

«سورة العلق، الآيات 1 – 5»

الإهداء

إلى من بلغ الرسالة وأدى الأمانة ونصح الأمة نبي الرحمة
نبينا محمد (صلى الله عليه واله وسلم)

إلى الرجل المثالي أطال الله في عمره ليظل عونًا لي.
(أبي)

إلى من وضع المولى سبحانه وتعالى الجنة تحت قدمها ووقرها في كتابه العزيز
(أمي)

إلى اساتذتي ممن كان لهم الدور الأكبر في مساندتي ومدي بالمعلومات القيمة.
إلى استاذتنا الدكتورة
(علياء معن عبد الحميد)
أهدي لكم بحث تخرجي....

داعياً المولى عز وجل أن يطيل في اعماركم ويرزقكم بالخيرات

الباحثون.

الشكر والتقدير

الحمد لله رب العالمين والصلاة والسلام على سيد المرسلين وخاتم الأنبياء والمرسلين سيدنا محمد (صلى الله عليه وعلى إله الطيبين الأطهار).

احمد الله تعالى حمداً كثير طيباً مباركاً ملئ السموات والأرض على ما أكرمني به من إتمام هذه الدراسة الذي أرجو أن تنال رضاه.

ثم أتوجه بجزيل الشكر وعظيم الامتنان إلى :
الدكتورة الفاضلة / علياء معن عبد الحميد حفظها الله ، لتفضلها الكريم
بالإشراف على هذه الدراسة ،وتكرمها بنصحنا وتوجيهنا حتى اتمام هذه الدراسة.
وأتقدم بالشكر والعرفان إلى عمادة كلية العلوم ورئاسة قسم التقانة الاحيائية

Abstract

Infertility is the inability to have children after two years of a normal sexual life without the use of any contraceptive method for both spouses. Female infertility is a disorder that occurs in women for many known or unknown reasons. The present study was taken up to find out Beta 2 Microglobulin (b2M) gene variants and their association with infertility . Furthermore, it showed the differences in some variables such as age, WBC, Hb, duration of infertility, and duration of period between the two groups. Forty five participants were selected for this study, 25 for were identified as infertile women and the rest were considered control. Blood samples were collected from the participants and WBC and Hb were measured.

DNA was extracted from the plasma and PCR was performed to amplify B2M gene, then an electrophoresis was performed to detect the DNA bunds using the specific primers. Gel electrophoresis was used to confirm the PCR results.

The results of the research showed that there was no significant difference between the B2M gene presence between the controls and infertile women. Similarly, there was no significant differences in the duration of period, the number of WBC , Hb values. However, it was a significant difference between the infertile women according to their ages and the number of infertile women with age 36 -40 were significantly higher (44%) than the rest . Whereas as the control did not show difference in the number according to the age group.

1. Introduction:

Infertility is the inability to have children after two years of normal sexual life without the use of any contraceptive for both spouses. Infertility in women is a state of disorders that occur in women, for many reasons that may be known or unknown. Infertility in women is divided as primary sterility that affects a woman since the beginning of her sexual life or marriage. Which is usually due to glandular or hormonal diseases, or the immaturity of the reproductive organs for the reasons for its formation. The rate of primary infertility is high in cold countries. Whereas, the secondary infertility affects a woman after giving birth to one or two children or after having an abortion. It is caused by complications of childbirth, miscarriage and all infections that may affect the uterus and nevus. Secondary relative infertility is high in developing countries.

β2 microglobulin, is a small membrane protein (11800 daltons) associated with heavy chains of major histocompatibility complex proteins of class I, and therefore it is present on the surface of all nucleated cells. The small size of beta-2-M allows it to pass through the glomerular membrane, but it is almost completely absorbed into the proximal tubules.

Blood levels of beta-2-M are elevated in diseases associated with an increased rate of cell division. Levels are also elevated in many benign conditions such as chronic inflammation, liver disease, renal impairment, some acute viral infections, and a number of malignancies, especially hematological malignancies associated with B-lymphocyte lineage-related.

1.2 The aim of the study

The present study was taken up to find out Beta 2 Microglobulin (b2M) gene variants and their association with infertility using conventional PCR and electrophoresis techniques.

Keywords:

Infertility women, B2m, PCR , Gel electrophoresis, and Plasma

1.3 Background

Infertility has been documented as a public health issue worldwide by the World Health Organization (WHO) [1] . Based on WHO criteria, infertility is defined as failure to achieve conception after 12 months or more of regular unprotected sexual intercourse . Infertility is classified as primary for a couple with no children, or as secondary after having one child. Based on a systematic review, the global estimate of infertility for non-surgically sterilized fertile women revealed a regional variation in infertility with a global estimation of 72.4 million infertile women worldwide, of whom 40.5 million are seeking fertility treatment. Moreover, estimation of the infertility based on 277 surveillance representing 190 different region, revealed that 48.5 million couples worldwide were unable to have a child after five years [2]. An epidemiological study of infertility in Scotland revealed that approximately one in five women had experienced infertility [3], and the authors concluded that the main factors associated with infertility were endometriosis, Chlamydia trachomatis infection and pelvic surgery. Moreover, the prevalence of infertility was estimated at 2.5% in the UK [4]. A subsequent study in 2009 estimated annual infertility at 0.9 among 1000 couples . The latest estimated infertility rate in the UK is one in seven couples , but there is considerable variation in the prevalence of infertility among different studies. This is probably because of differences in the definition of infertility and in measurements used. For instance, one study was conducted in infertile couples who had experienced 5 years of infertility [5] and in comparison in another study the inclusion criteria stipulated that any infertile couple had to have been attempting conception for 24–48 months .The epidemiology of infertility is based on those infertile couples who seek help. Basically, the main overall causes of female infertility are ovulatory disorders, with an incidence ranging between 21–32%, and tubal disorders which vary between 14–26% .

1.4 Female-Fertility and the Immune System

A comparison of the immune and female reproductive systems would appear, at first glance, to be a juxtaposition of two highly divergent systems. One involves a highly diverse repertoire of cell types functioning throughout a wide range of tissues to detect and fight infection and disease, while the other is dedicated to the development of a highly specialized haploid gamete upon which fertilization is dependent. However, both of these systems, at their most fundamental level, are reliant upon a critical cellular mechanism: the ability to recognize specific cell types with incredible specificity in an environment composed of a myriad of other cell types[7]. In the case of the immune system, this often involves the recognition, marking, and destruction of foreign, non-host cells without inappropriately targeting the diverse repertoire of host cells the system is likely to encounter. In the context of the female reproductive system, sperm have evolved to traverse a wide range of tissues within both the male and female reproductive tracts, encounter an array of different cell types and specifically recognize, bind, and fuse with one of the very limited number of oocytes present. This fundamental shared molecular capability might lead one to predict that the machinery responsible for cell-cell recognition in these systems are related, either at the level of individual proteins or pathways[8].

Immunology of the female reproductive tract

The epithelium and mucosal immune system in the female reproductive tract provides protection against pathogens while also accommodating the requirements of reproduction, namely tolerance of sperm and the resulting fetus [9].The epithelium secretes a variety of antimicrobials, including complement, and antibodies such as IgA, IgG, and IgM [10]. The heightened potential of immune responses within the female reproductive tract is likely associated with the prevalence of infection, some of which may be directly related to the transmission of sexually transmitted diseases . Bacteria can be commonly identified in human semen , although the impact of any resulting female immune response on sperm quality is not well understood [11] .

1.5Causes of female infertility:

Causes of female infertility may include:

- **Ovulation disorders**, which affect the release of eggs from the ovaries. These include hormonal disorders such as polycystic ovary syndrome. Hyperprolactinemia, a condition in which you have too much prolactin — the hormone that stimulates breast milk production — also may interfere with ovulation. Either too much thyroid hormone (hyperthyroidism) or too little (hypothyroidism) can affect the menstrual cycle or cause infertility. Other underlying causes may include too much exercise, eating disorders or tumors (Fig.1).
- **Uterine or cervical abnormalities,** including abnormalities with the cervix, polyps in the uterus or the shape of the uterus. Noncancerous (benign) tumors in the uterine wall (uterine fibroids) may cause infertility by blocking the fallopian tubes or stopping a fertilized egg from implanting in the uterus.
- **Fallopian tube damage or blockage**, often caused by inflammation of the fallopian tube (salpingitis). This can result from pelvic inflammatory disease, which is usually caused by a sexually transmitted infection, endometriosis or adhesions.
- **Endometriosis**: which occurs when endometrial tissue grows outside of the uterus, may affect the function of the ovaries, uterus and fallopian tubes.

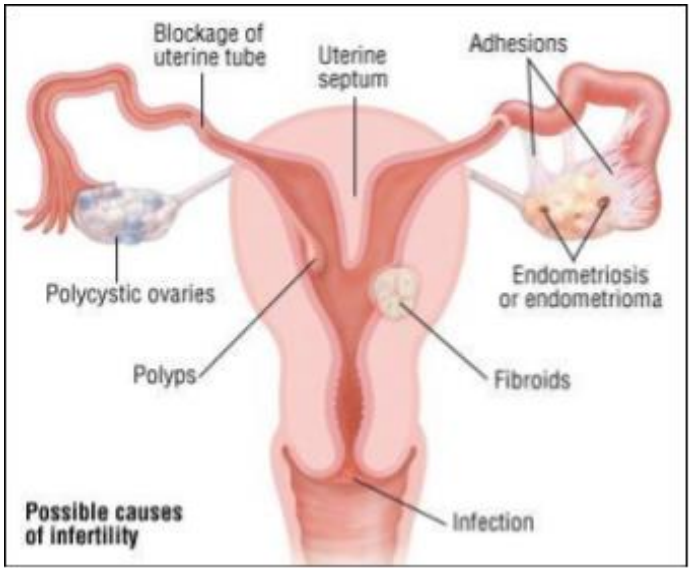

Figure 1: female infertility

There are many other risk factors for fertility problems in women, including:

- **Age**: Women's fertility gradually declines with age, especially in the mid-30s, and it drops rapidly after age 37 (Fig 2). Infertility in older women is due to the lower number and quality of eggs, and can also be due to health problems that affect fertility. Men over age 40 may be less fertile than younger men[12].

- **Tobacco use**: Smoking tobacco or marijuana by either partner may reduce the likelihood of pregnancy. Smoking also reduces the possible effectiveness of fertility treatment. Miscarriages are more frequent in women who smoke. Smoking can increase the risk of erectile dysfunction and a low sperm count in men.
- **Alcohol use:** For women, there's no safe level of alcohol use during conception or pregnancy. Alcohol use may contribute to infertility. For men, heavy alcohol use can decrease sperm count and motility.

- **Being overweight:** Among American women, an inactive lifestyle and being overweight may increase the risk of infertility. For men, sperm count also may be affected by being overweight.

- **Being underweight**: Women at risk of fertility problems include those with eating disorders, such as anorexia or bulimia, and those who follow a very low-calorie or restrictive diet.

- **Exercise issues**: A lack of exercise contributes to obesity, which increases the risk of infertility. Less often, ovulation problems may be associated with frequent strenuous, intense exercise in women who are not overweight.

- **Diabetes** ;There are many reasons behind infertility, and diabetes can be one of these reasons, but the test is what determines if glucose levels are the main reason for this, and type 2 diabetes is one of the conditions that can impede the chances of getting pregnant, and may really cause infertility in men. As for type 1 diabetes, it can cause many problems for both the mother and the fetus, and in the case of second degree diabetes, precautionary measures must be taken to obtain a safe pregnancy, and there are some facts that must be aware.

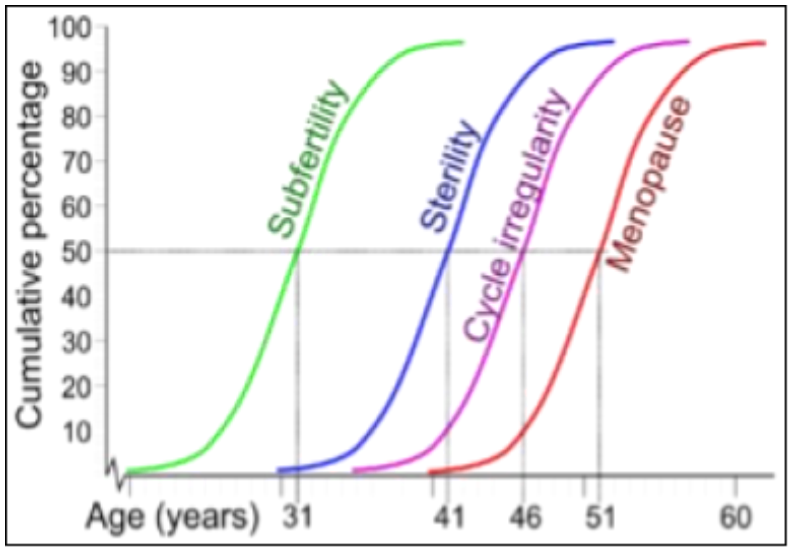

Fig.2 the age average of infertility[6, 10]

1.6 The causes of infertility from microorganisms

Bacteria

Chlamydia is one of the sexually transmitted, and this parasitic bacteria infects the reproductive system in men and women with the same ability to destroy the reproductive system without clear symptoms, and it may cause infertility for both sexes, and the infection is often asymptomatic. It is one of the most common venereal diseases and it is a bacterium that has the ability to destroy the reproductive system without clear and apparent symptoms, and in many cases the affected organs cannot return to their normal function, which may lead to infertility, especially in women before they realize that they have been infected[13]. tract infections affect a woman's ability to conceive, including inflammation of the cervix, vagina and ovaries, and microbial infection is the main cause of genital tract infection, most notably fungi and herpes syphilis (Fig. 3).

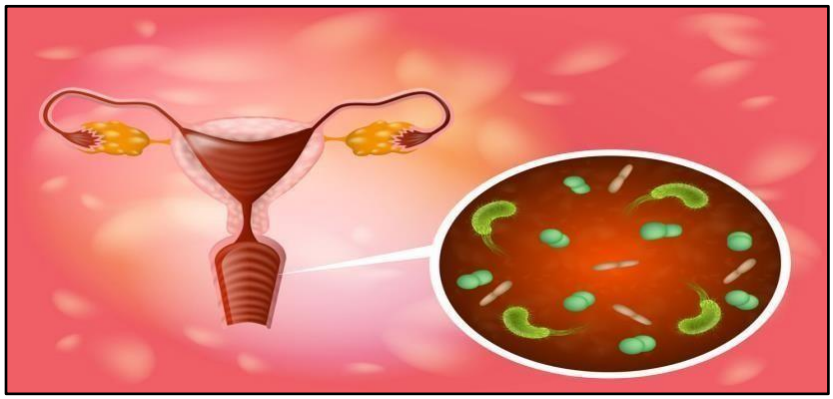

Fig.3 the types of vaginal bacterial infection [6]

Virus

A team of scientists discovered a mysterious virus that is one of the most important causes of herpes disease. It is possible that the endometrium becomes unexplained infertility among women. The recent study conducted by scientists from the Italian University of Ferrara indicated that the reason behind the inability to conceive for some A human herpesvirus that lives in the mucous membrane in the uterus of the woman and prevents the completion of pregnancy, as scientists did not find this virus in the uterus of women who could conceive, and the scientists found a relationship between the human herpes virus 6 (or (HHV-6A) . samples of the human herpes virus 6, i.e. "in the reproductive system of women, because this virus is not considered by scientists to be widespread, and the virus cannot be identified in blood samples or saliva samples.

The researchers considered that there is a close relationship between the virus and the levels of the hormone "estradiol" that is secreted by the ovaries in females and responsible for the maturation of the egg, and that the response of the immune system to the virus could contribute to making the uterus less hospitable to the fertilized egg, and scientists advised to conduct more in-depth studies. To know the nature of this relationship and its effects on the inability to conceive .

Fungi

Fungal vaginal infections or Candidiasis usually occurs in women. The vagina usually contains fungal cells and bacteria. Fungi (candidiasis) are microorganisms that are found naturally in the vagina. Lactobacillus is responsible for monitoring their growth. But if the two are not balanced, the fungal cells may begin to proliferate and lead to infection.

Candida albicans, in particular, is the type of yeast vaginitis that results in most yeast infections.

Fungal vaginitis can lead to intense itching, irritation and swelling. Another symptom is a lumpy, gray-white vaginal discharge. Factors such as antibiotics, uncontrolled diabetes, a poor immune system, pregnancy, hormonal imbalance, or stress can trigger an active yeast infection.

Fungal. There is no conclusive evidence available to suggest that yeast infection causes infertility in women. However, the set of symptoms associated with a yeast infection that includes soreness, swelling, irritation, and burning in the vagina can make having sex uncomfortable and painful. Therefore, a woman with a yeast infection may not be tempted to engage in sex[14]. Therefore, a yeast infection does not directly affect fertility but it can contribute to pregnancy problems by interfering with her sexuality indirectly. Recurring vaginal yeast infections can make the vagina hostile to sperm retention, making pregnancy difficult and creating conditions for candida sterility [15].

1.7 Beta-2-microglobulin (B_2M)

In 1968, Beggard and Bearn first isolated beta2-microglobulin (β2-m) from urine of patients with renal proximal tubule disorders. β2-m is a small membrane protein that is highly stable during evolution; it is encoded in the sixth chromosome. Beta2-microglobulin is composed of 99 amino acids; due to one peptidic bound it creates a loop. It belongs to the immunoglobulin superfamily, and its primary and secondary structure is strikingly similar to IgG structure; thus, it is suggested to arise from the same ancestral gene[16].

Beta 2 macroglobulin also known as B2M is a component of MHC class I molecules, MHC class I molecules have α1, α2, and α3 proteins which are present on all nucleated cells (excludes red blood cells). In humans, the B2 microglobulin protein is encoded by the B2M gene. B2M protein found in all nucleated cells is associated with histocompatibility class 1 antigens on cell surface membranes (particularly abundant on lymphocytes and monocytes).

It was first discovered in the urine of patients with renal failure. Because of its low molecular weight (11,800 daltons), 95% of all free B2M in plasma is eliminated by glomerular filtration, and 99.9% of it taken up by proximal tubular cells. In the presence of a normal renal filtration rate, elevated serum B2M (Sb2M) levels indicate high B2M production or release[17].

1.8 Hb measurement and correlation with infertility

Hemoglobin (Hb) is the protein molecule in red blood cells that carries oxygen from the lungs to the body's tissues and returns carbon dioxide from the tissues back to the lungs. Research into hemoglobin has been conducted for decades in red blood cells, but little is known about this protein anywhere else in the body.

In the ovary, the level of internal hemoglobin continues to rise in the oocyte and its surrounding cells, otherwise known as the cumulus oocyte complex, as it matures. "The complication with this project is that we know very little about the role of hemoglobin outside of red blood cells. However, given that hemoglobin is really important for carrying oxygen we think this is the main role its playing inside the oocyte, and it is especially important given the possible low oxygen environment of the ovary and reproductive tract

The ovary is very unique in terms of its oxygen biology, and the oocyte grows in an environment that has little access to blood vessels and direct oxygen. A study that explained how and when hemoglobin was produced in the ovary during preparation for ovulation. They found the two components - alpha and beta hemoglobin - that likely make up functional hemoglobin.

Both components increase dramatically in the time leading up to ovulation, before the egg travels through the fallopian tubes to meet the sperm.

25 samples were collected from women (19 samples from infected women with infertility and 6 samples from normal women) and it was found that there is no relationship between hemoglobin and fertility

1.9 WBC and correlation with infertility

White blood cells (WBCs), are one of the major blood cells in addition to the red cell and platelets. The main function of these cells is to defend the body against infectious diseases and against microbial invasion, and they are part of the immune system. White blood cell count is often an indicator of disease. There are usually 4,000-11,000 white blood cells in a microliter of blood, which is about 1% of the blood of a healthy adult. When the body is under attack by antigens, this number increases slightly. In cases such as leukemia, the number of white blood cells is higher than normal, and in leukopenia, this number is much lower. An elevated WBC may be an indication of a viral or bacterial infection, which is one of the causes of infertility. Considering the percentage of WBC and its relationship in Infertility A comparison was made between WBC and infertility

25 samples were taken 6 Patients 19 normal.

There were no statistically significant differences between infertile females or normal furniture and WBC, all values fall within a normal range as shown

Material and working methods

2. Materials used

2.2 Laboratory equipment and supplies

The were used the laboratory equipment and supplies listed in Table (1) in the current study

Table(1) : Laboratory equipment and supplies used in the study

Laboratory equipment	Manufacture company
(BSC _ ll)	Kimo, 24700 Montpon, France
Oven	Memmert, Germany
Centrifuge cooling	Sigma 1_ 13,Harz, Germany
High speed cold centrifuge	Germany Memmert
Vortex mixer	Sigma 1_13, Harz, Germany
PH meter	Biomax, Desage, Heidelberg, Germany
Sensitive balance	Sartorius, Germany
Refrigerator	Diora , Turkey
Nanodrop spectrophotometer	Thermo, U. S. A
Thermal Cycle	Alpha DNA, UK
Electrophoresis set	Helena, USA
UV _transillminatotou	Herolab, Germany
Gel documentation	Herolab, Germany
Micropipettes	Brand, Germany
Magnetic stirrer	China
DdH2O	Lab. Tech, Korea
Screw _ capped	Schott, Germany
Flasks	Schott, Germany

2.3 : Chemicals used

The use of the chemicals shown in the table (2) during the study period

Table 2 :The chemicals used in the study

The substance	The manufacture company
Agarose	Promega, USA
Ethidium Bromide	Promega, USA
TBE _ buffer (10x)	Promega, USA
Loading dye	Promega, USA
DNA Ladder 100bp/1000bp	Promega, USA

2.4 Participants and sampling

Blood samples were collected from among 45 volunteers who attended Al-Batool Teaching Hospital, Al-Naba Laboratory and Al-Shams Laboratory in Baquba, Diyala Governorate Center between November 20, 2020 to January 20, 2021 for 25 women (18 to 39 years old): consisting of 20 healthy subjects who considered as control and the rest are infertile. The period of sample collection (day 12 or 13 of the menstrual cycle) and delivery to the laboratory using test tubes, the plasma was isolated from the rest of the blood components using a centrifuge and then kept in the freezer.

2.5 WBC and Hb measurements

Blood samples were collected in tubes containing anticoagulant - using tubes containing EDTA (ethylenediaminetetraacetic acid) to prevent normal blood clotting. Blood was drawn from a vein. Then conduct the test using an automated analyzer.

The results of the examination indicate that a decrease or increase in the number of white blood cells leads to a disorder in the blood or the presence of severe infections or other viral infection, which may be one of the causes of infertility.

A low level of HB leads to anemia, while a high percentage of red blood cells leads to a high level of HB, which can be one of the causes of infertility.

After conducting an Hb and WBC test, it was found that all percentages fall within the normal range

For normal and infertile women.

Normal Hb range for women

(12 _ 15 g/dl)

The normal WBC range for women

(4000 _ 11,000 cells per μl).

So, there are no statistically significant differences between sterile and normal females for WBC and Hb**.**

2.6 DNA extraction from blood

All blood samples were preserved. Plasma was isolated from blood cells, ready for immediate DNA extraction, and kept at -20°C. DNA was extracted according to the instructions of the DNAMini ® QIAamp, which takes 20 minutes**.**

1. 20 µL QIAGEN Protease (or Proteinase K) was placed at the bottom of a 1.5 ml microcentrifuge tube by means of a mechanical pipette.

2. Add a 200 µl sample to a microcentrifuge tube. 3

. 200 µl AL buffer was added to the sample. Mix by vortex mixer for 15 seconds. To ensure effective lysis, it is essential that the sample and Buffer AL are thoroughly mixed to give a homogeneous solution**.**

4. Samples were incubated at 56°C for 10 minutes. DNA production reached a maximum after lysis for 10 minutes at 56°C.

5. The sample was briefly centrifuged into a 1.5 ml microcentrifuge tube to remove droplets from the inside of the cap**.**

6. Add 200 µl of ethanol (96-100%) to the sample, and mix again by vortex for 15 seconds. After mixing, the 1.5 ml microcentrifuge tube is briefly centrifuged to remove droplets from inside the cap.

7. The mixture was carefully applied from step 6 to the spin columeQIAamp Mini spin column (in 2 ml .).

collecting tube) without wetting the tip. The lid was closed, and placed in the centrifuge at 6000×g

(8000 rpm) for 1 minute. The spin column QIAamp Mini was placed in a clean 2 mL tube, and the tube containing the filter was discarded. *Close all spindles

Centrifugation was performed at 6000 × g (8000 rpm**).**

8. The spin columnQIAamp Mini was carefully opened and 500 µL of buffer AW1 was added without wetting the tip. The lid was closed and centrifuged at 6000 × g (8000 rpm) for 1 min. The QIAamp Mini spin column was placed in a clean 2 mL collection tube (supplied), and

Discard the collecting tube that contains the filter. *****

9. The QIAamp Mini spindle is carefully opened and we add 500 µL AW2 without wetting the tip. Close the lid and centrifuge at full speed (20000 × g;14,000 rpm) for 3 min.

10. Put the QIAamp Mini spindle into a new 2ml collection tube and discard the old collection tube with the filter. Place in centrifuge at full speed for 1 min.

This step helps get rid of Buffer AW2.

11. Put the QIAamp Mini spindle into a clean 1.5ml microcentrifuge tube and carefully open Spindle QIAamp Mini and add 200 µL of buffer AE or distilled water.

Incubate at room temperature (15-25°C) for 1 minute, then centrifuge

6000 x g (8000 rpm) for 1 minute**.**

Detection of the Beta-2-microglobulin (β2-M) gene:

2.7 B2M measurement using gene detection

The concentrations of DNA extracted from serum samples (45 samples) , purity was measured based on absorbance values 260/280 as it ranged between 1.43- 0.79. All samples are consecutively numbered by electrophoresis through the detection of special β2M sequences of the selected gene characteristic

The interaction of its size is 105 base pairs as noted in Figure (4), and it has been proven through the results that all samples used in this study belong to β2M sequence amplification used to confirm its presence in clinical samples Li Eirini Neofytou et.al, 2009. Where his study agreed tagged.

2.8 PCR Method for Amplification of Beta-2-microglobulin (β2-M):

DNA extracting from Blood**.** The b2microglubulin sequences were downloaded from the GenBank sequence database of the National Center for Bioinformatics NCBI, using Primer3 program. The primers were manufactured by Koma Biotech Inc. (South Cori**a).**

B2m (forward)	B2m(reverse)
5_TCC AAC ATC AAC ATC TTG T_3F	5_ TCC CCC AAA TTC TAA GCA GA _3R

Figure 4: The B2M primer

Preparation of PCR reaction

Forward and reverse primers which in lyophilization status were dissolved and diluted first in free nuclease D.S.D.W(amount according to recommended of manufactured company) to obtains 100 pico-mol/µl and this is considered as a stock solution, then it can be stored in deep freeze. This stock was diluted in free nuclease D.D.W to obtain nearly 10 pico-mol/µl and stored in deep freeze until used in PCR mixture.This technique accorded with all primers in this study, as listed in table**.** The components of the polymerase chain reaction and the quantities that were mixed are shown in Table (4 & 5)

Table 4 : Protocol of PCR reaction mixture volumes used in the current study.

PCR reaction	Volume (µl)
PCR Master mix	10µl
DNA template	5µl
Forward primer (10 pmol)	1µl
Reverse primer(10 pmol)	1µl
ddH20	3µl
Total volume	20 µl

The reaction components mentioned above are placed in the tubes in the standard kit, which contains all the other components needed for the reaction (Tag DNA Polymerase, Tris-Hcl PH 9.0, dNNTPs, KCL, Mgcl2, Stabilizer, tracing dye PCR) and all the reaction tubes were mixed by Vortex At a speed of 3000 rpm for a period of 3 minutes, then it was placed in a PCR thermocycler using the Convential PCR thermocycler system for each gene (Figure5).

Table 5 : Protocol of PCR reaction mixture volumes used in the current study**.**

PCR programe	**Temperature (°C)**	**Time**	**Number of cycle**
Initial Denaturation	**94**	**5min**	**35**
denaturation	**94**	**30sec**	
Annealing	**53**	**30sec**	
extension	**72**	**30sec**	
Final extension	**72**	**10min**	
Hold	**4**	**5min**	

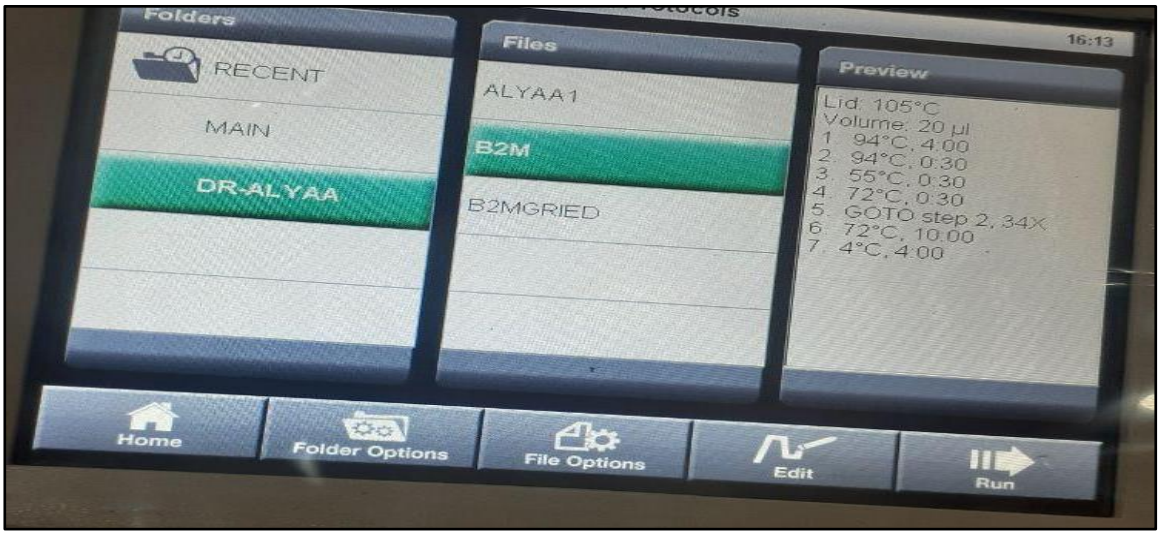

Figure 5 : Programs of PCR thermocycling conditions for detection of

2.9 Preparation of agarose gel and electrophoresis:

A agarose gel was prepared at a concentration of 2% by dissolving 1 g of agarose powder in 50 ml of 1X TBE buffer. The solution was heated to boiling and the solution was homogenized to a clear form, and then allowed to cool at 50°C, 1 microliter of 0.3mg/ml ethidium bromide dye was added to the solution and mixed well; So gently tower the solution.. The mixture is poured into a tray (to which the Combo is pre-installed) for the holes needed to load the DNA samples, left at room temperature to solidify for 45 minutes, the hardened gel is transferred to a gel tank and the comb is raised quietly, and placed Under the transfer basin is an opaque black plate so that the pits are clearly visible and the transfer basin is filled with 1X TBE buffer until it covers the gel. DNA samples are placed in the holes using a micropipette with a volume of 10 microliters from each sample, taking into account that the sample does not come out from the surface of the hole. The DNA ladder is placed in the hole designated for it on one side of the gel with a volume of 7 microliters and mixed with 3 microliters of buffer loading. Then, the process of electrophoresis is carried out by connecting the electrodes and preparing it with a capacity of 65 volts, and the relay is carried out towards the positive electrode. After 45 minutes, when the blue dye reaches the end of the gel, the relay is stopped and the gel is transferred to the UV Trans-illuminator (Figure 6). At a wavelength of 320 nm to visualize the DNA bundles and estimate its molecular size in comparison with the volume guide, photograph the gel using an imaging device (Sambrook and Russel, 2001).

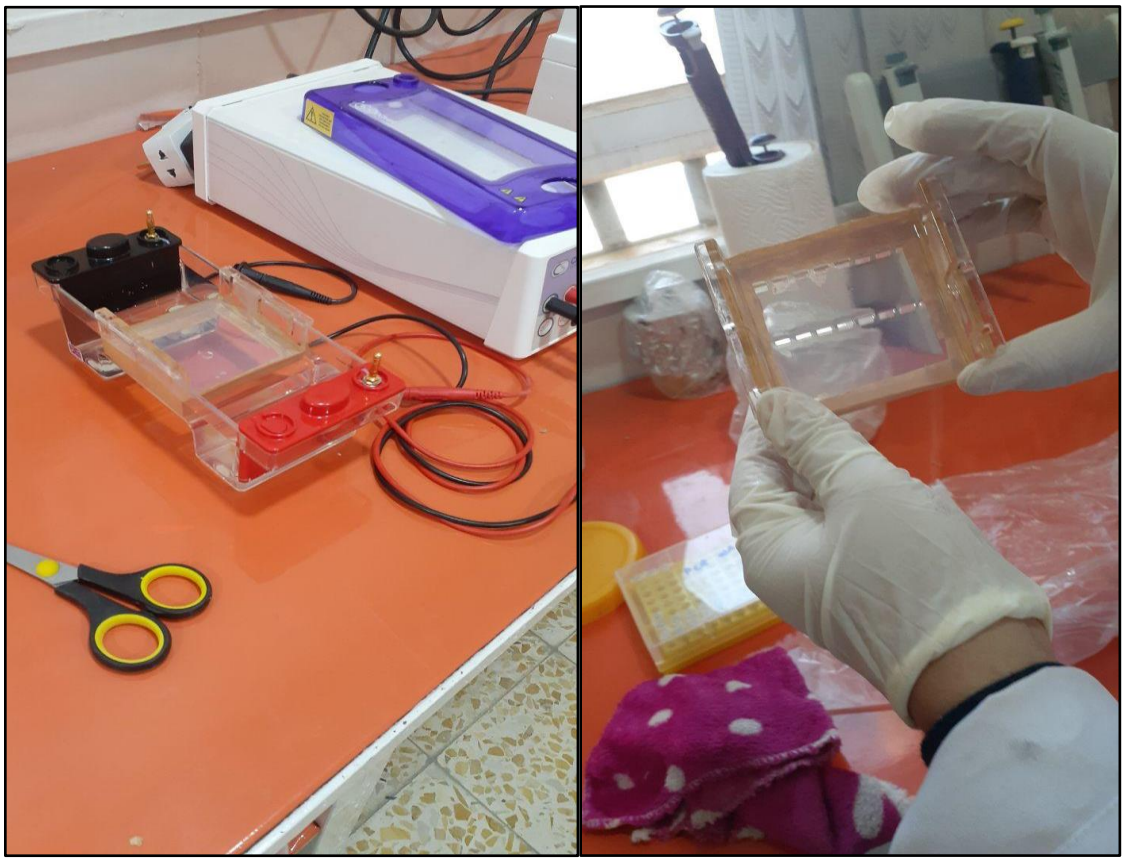

Figure 6: Gel electrophoresis for PCR product by using B2M primers

3. Results and discussion

The results of the research in the table are presented in table (6) showed the significant (*P value*<0.05) differences at age and level between infertile women and control. However, it was a significant difference between the infertile women according to their ages and the number of infertile women with age 36 -40 were significantly higher (44%) than the rest . Whereas as the control did not show difference in the number according to the age group. In contrast, other study found no significant differences between infertile and control participants depending on age group (Jasim M et al, 2019) .

Table 6 : The age distribution of participants

Age period of study groups				
Age periods	Age group		Groups of Participants	
			Control	Infertile
	15-20	N	1	1
		%	5%	4%
	21-25	N	3	*5
		%	15%	20%
	26-30	N	6	3
		%	30%	*12%
	31-35	N	6	5
		%	30%	*20%
	36-40	N	4	11
		%	20%	*44%
Total		N	20	25
		%	100%	100%
P-values		*P value*>0.05		*P value* ≤0.05

In this study there was no a significant differences between the number of infertile women and control (Table 7).

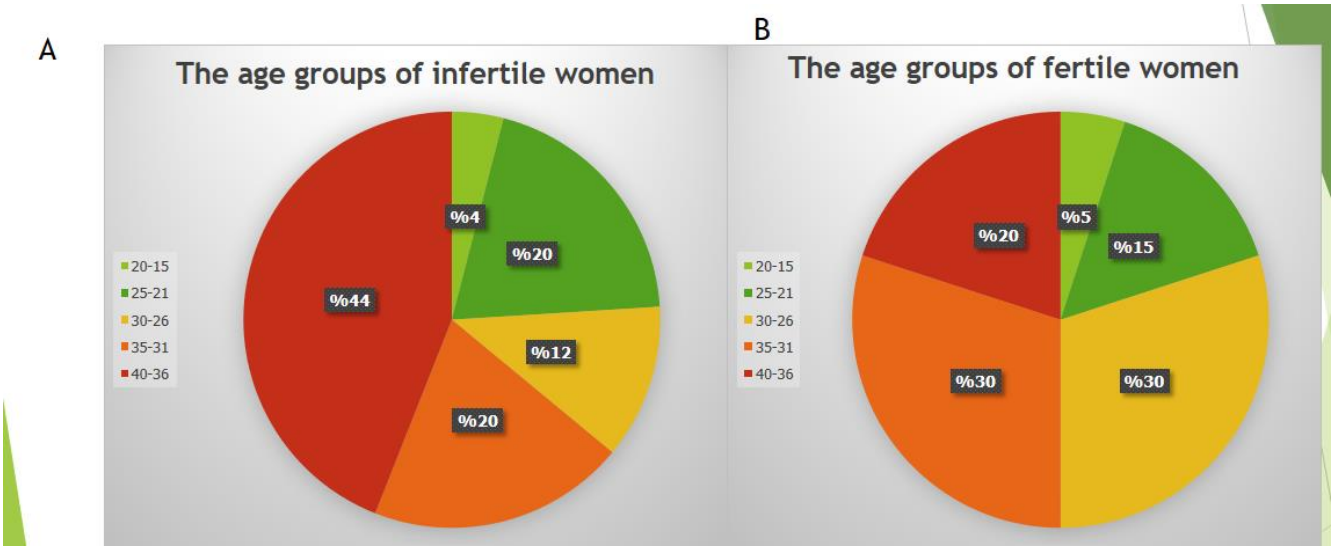

Figure 7A: The infertile women age groups :B the fertile women age group

Table 7: The study groups in current research

Study groups	**No**	**Percentage (%)**	P-value
Infertile	25	44.5%	P >0.05
Control	20	55.5%	
Total	45	100%	

The an extraction of genomic DNA from 45 samples was confirmed as bands by gel electrophoresis. DNA concentration was determined using the Quantus Fluorometer. All samples showed positive bands for B2M for both infertile and controls (Figure 7).

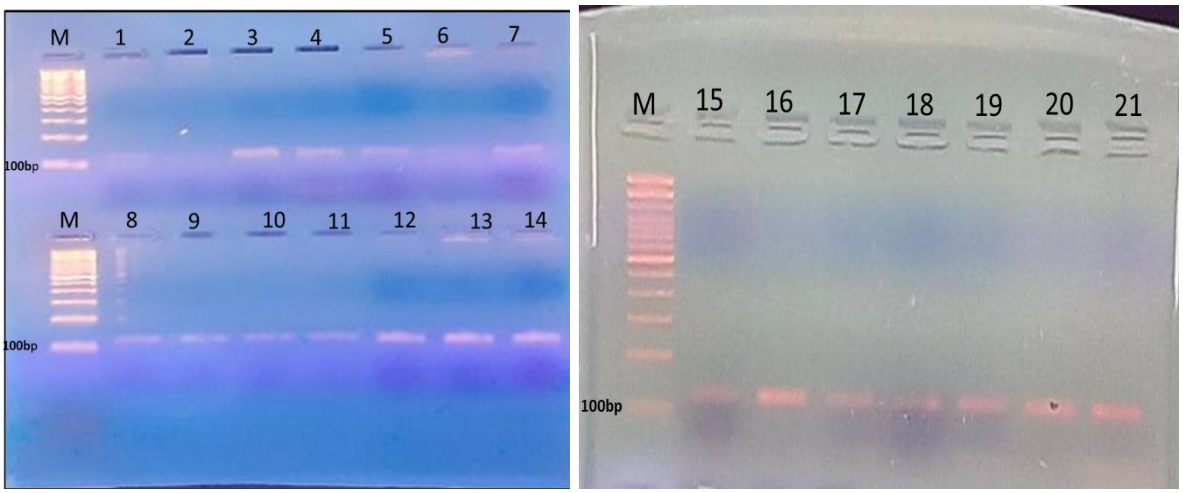

Figure 8: Gel electrophoresis of amplified PCR product for the detection of B2M (105 bp) run on 1%agarose (90 min at 70 volt), stained with ethidium bromide, lane 1-21 ; M:Marker DNA ladder (100bp); All lanes are positive .

The results are conducted in this study showed no significant differences in blood variables between that the infertile women and controls. Table 8 showed normal values for all blood variables in the infertile and control for all the participants. It was found that there are no statistically significant differences between females with infertility or control . WBC and Hb values are within the normal range as shown, noting that the normal ratio WBC 11000 and 12.5, respectively. Similarly, the duration of period did not show a significant difference ($p>0.05$). However, there is significant differences between the duration of infertility according to the age group (p value <0.05). (20)

Table 8: The comparison between infertile women and controls using some variables

Variables	Control			Infertile			T-test	P-value
	Mean	Std. Deviation	Std. Error Mean	Mean	Std. Deviation	Std. Error Mean		
WBC	**6.8**	**1.5**	**0.3**	**6.7**	**1.3**	**0.2**	0.2525	P > 0.05
B2M	**1**	**0**	**0**	**1**	**0**	**0**	0	P > 0.05
Hb	**12.7**	**1.4**	**0.2**	**12.6**	**1.2**	**0.2**	0.1894	P > 0.05
Duration of infertility (year)	**1.5**	**3**	**0.6**	**5**	**3**	**0.6**	2.209	***P < 0.05**
Duration of period (day)	**5**	**1.2**	**0.2**	**5**	**0.9**	**0.1**	0.2525	P > 0.05

1. Beck, S., et al., *The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity.* Bulletin of the World Health Organization, 2010. **88**: p. 31-38.
2. Ghuman, N. and M. Ramalingam, *Male infertility.* Obstetrics, Gynaecology & Reproductive Medicine, 2018. **28**(1): p. 7-14.
3. Zhou, Z., et al., *Epidemiology of infertility in China: a population-based study.* BJOG: An International Journal of Obstetrics & Gynaecology, 2018. **125**(4): p. 432-441.
4. Den Heijer, C.D., et al., *Chlamydia trachomatis and the risk of pelvic inflammatory disease, ectopic pregnancy, and female infertility: a retrospective cohort study among primary care patients.* Clinical Infectious Diseases, 2019. **69**(9): p. 1517-1525.
5. Mascarenhas, M.N., et al., *Measuring infertility in populations: constructing a standard definition for use with demographic and reproductive health surveys.* Population health metrics, 2012. **10**(1): p. 1-11.
6. Roy, K.K., et al., *Reproductive outcome following hysteroscopic myomectomy in patients with infertility and recurrent abortions.* Archives of gynecology and obstetrics, 2010. **282**(5): p. 553-560.
7. Prieto, L.I., S.I. Graves, and D.J. Baker, *Insights from in vivo studies of cellular senescence.* Cells, 2020. **9**(4): p. 954.
8. Neubig, R.R., et al., *Mechanisms of Cellular Signal Transduction.* Cellular Signal Transduction in Toxicology and Pharmacology: Data Collection, Analysis, and Interpretation, 2019: p. 21-48.
9. Lessey, B.A. and S.L. Young, *Structure, function, and evaluation of the female reproductive tract*, in *Yen and Jaffe's Reproductive Endocrinology*. 2019, Elsevier. p. 206-247. e13.
10. Chen, K., et al., *Rethinking mucosal antibody responses: IgM, IgG and IgD join IgA.* Nature Reviews Immunology, 2020. **20**(7): p. 427-441.
11. Baud, D., et al., *Sperm microbiota and its impact on semen parameters.* Frontiers in microbiology, 2019. **10**: p. 234.
12. Tozzo, P., et al., *Understanding social oocyte freezing in Italy: a scoping survey on university female students' awareness and attitudes.* Life sciences, society and policy, 2019. **15**(1): p. 1-14.
13. Rowe, M., et al., *The reproductive microbiome: an emerging driver of sexual selection, sexual conflict, mating systems, and reproductive isolation.* Trends in ecology & evolution, 2020. **35**(3): p. 220-234.
14. Bautista, C.T., et al., *Bacterial vaginosis: a synthesis of the literature on etiology, prevalence, risk factors, and relationship with chlamydia and gonorrhea infections.* Military Medical Research, 2016. **3**(1): p. 1-10.
15. Kanchana, C., *Formulation, Characterization and Evaluation of Nystatin Nanosponge GEL For the Treatment of Candidiasis*. 2016, College of Pharmacy Madras Medical College, Chennai.
16. Klatt, E.C., *Pathology of HIV/AIDS*. 2019, Savannah: Mercer University School of Medicine.
17. Filippin, F.B. and L.C. Souza, *Serum beta2-microglobulin values among healthy Brazilians using a DPC IMMULITE® assay.* Clinics, 2005. **60**(1): p. 47-50.
18. Eirini N, George S., Maria A.,(2009) Prevalence of human herpes virus types 1–7 in the semen of men attending an infertility clinic and correlation with semen parameters. Fertility and Sterility.Vol. 91,

19. JasimM. M. , Sura O, Ameer M. Had*, Yousif and Mohammed Haider HamadMale(2019). Infertility and Viral Infection: Interference Role of the Human Herpesvirus types (3 – 6) with Disturbances Effects of Some Cytokines Hypersecretion and Seminal Oxidative Defense System in the Infertility Etiopathogenesis of Some Idiopathic Infertile Iraqi Patients. Biomedical & Pharmacology Journal, .Vol. 12(3): 1181-1192.

20.(CellDynReferenceBooklet;AbbottDiagnos