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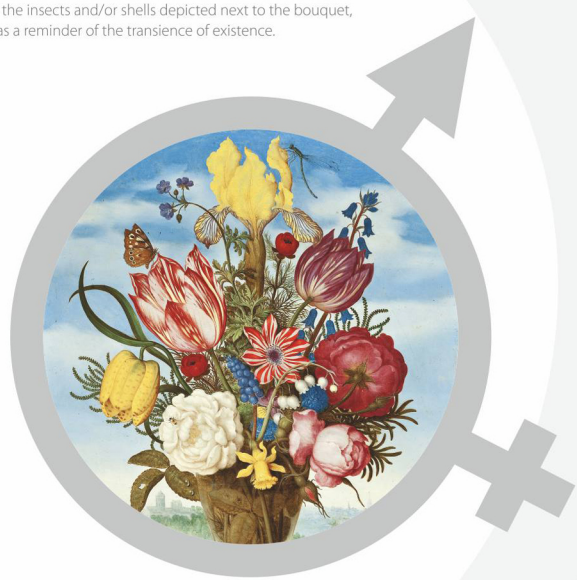
Bouquet of Flowers on a Ledge. Circa 1620.A work by Ambrosius Bosschaert the Elder (1573-1621).
This Flemish artist became famous for his naturalistic flower still lifes.
A characteristic feature of his paintings is the insects and/or shells depicted next to the bouquet,
as well as flowers touched by withering, as a reminder of the transience of existence.



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Effect of Acyclovir on Physiological Parameters and Reproductive Efficiency in Adult Male Rats (Rattus Norvegicus)

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Abstract

Introduction. Acyclovir is a synthetic analog of purine nucleoside that exhibits inhibitory activity against both herpes simplex virus types 1 (HSV-1), 2 (HSV-2), and varicella-zoster virus.
Purpose. To assess the impacts of varying doses of acyclovir on physiological parameters like liver enzymes, renal function and on reproductive efficiency by determination properties of semen, and some hematological parameters.
Materials and methods. The study utilized a sample of "twenty-four adult male rats (Rattus norvegicus) divided into four experimental groups of six rats each. The first group served as the control group, while the second group received a dosage of 50 mg/kg of acyclovir. The third group was treated with 100 mg/kg of acyclovir, and the fourth group received 150 mg/kg of acyclovir". These dosages were administered orally to the rats daily throughout the two-week duration of the experiment.
Results. A significant decrease in sperm count, severe and individual motility, and a strong association between reproductive efficiency and acyclovir in rats treated with acyclovir and induced oligospermia, in comparison to the control group. In the latest findings, a remarkable upsurge was detected in urea, creatinine, and liver enzymes like ALT, AST, and ALP levels among all the groups that were given acyclovir compared to the control group.
Conclusion. Astenozoospermia and teratozoospermia are commonest seen in semen analysis after acyclovir consumption. A significant decrease of sperm count and elevated of sperm abnormality are the major adverse effect of acyclovir. The presence of thrombocytopenia, leucopenia, a reduced count of red blood cells and platelets, and a decline in hemoglobin levels in all the rats treated with acyclovir.
Keywords: acyclovir, rats, physiological parameters, semen analysis, asthenozoospermia, teratozoospermia

INTRODUCTION

Acyclovir is a synthetic analog of purine nucleoside that exhibits "inhibitory activity against both herpes simplex virus types 1 (HSV-1), 2 (HSV-2), and varicella-zoster virus" [1] both in vitro and in vivo. Acyclovir is considered the primary treatment option for HSV encephalitis and is widely recognized as one of the most commonly prescribed antiviral drugs for this condition. No other indicated medications for treating HSV encephalitis [2]. Acyclovir functions as an antiviral agent by incorporating itself into the viral DNA, thereby impeding subsequent synthesis. The mechanism of action involves the inhibition of DNA synthesis and viral replication subsequent to the conversion of the compound into acyclovir triphosphate, which is facilitated by both viral and cellular enzymes [1]. Furthermore, the administration of acyclovir can be done orally to target mucocutaneous lesions specifically. However, intravenous administration of acyclovir is recommended in cases of widespread visceral or central nervous system involvement [3].

An acute kidney injury (AKI) is a notable adverse outcome associated with administering parenteral acyclovir, akin to the nephrotoxic effects of other medications such as aminoglycosides, as reported by [4]. The clinical application of acyclovir is restricted owing to its potential for inducing severe nephrotoxicity, despite its efficacy in treating diverse viral infections [5]. A lesser-known side effect of acyclovir administration is neurotoxicity which was demonstrated by the appearance of psychiatric symptoms [6]. Besides, acyclovir is a cytotoxic antiviral drug that causes apoptosis in the components of the reproductive system [7]. Exposure to environmental factors like irradiation and antiviral drugs can lead to DNA damage within cells, which induces apoptosis in germ cells and cytotoxicity in spermatogenesis [8].

MATERIALS AND METHODS

Animals and Drug Treatment

The study was carried out at the animal facility located within the premises of the College of Veterinary Medicine, University of Basrah. The study employed a sample of 24 adult male Wistar rats, aged 15 to 18 weeks, with an average body weight of 225±25 grams. The rodents were accommodated in a controlled environment with consistent parameters, such as a temperature range of 24–28 °C, a 12-hour light-dark cycle facilitated by fluorescent lamps, and a humidity level of 50%. A one-week of acclimatization period was provided to the rats prior to the commencement of the experimental study. The rats were accommodated in polyethylene cages that were equipped with wire mesh, and each cage was occupied by six rats. Throughout the experiment, the rats were provided with unrestricted access to commercial feed pellets and drinking water. Distilled water was utilized to dissolve Acyclovir to produce varying doses (50 mg/kg, 100 mg/kg, and 150 mg/kg) that were determined based on the body weight of individual rats. The rodents were allocated into four groups using a randomization process. The initial cohort was assigned to the control group, which was administered solely with water and sustenance. Acyclovir was administered to the second group at a dosage of 50 mg/kg of body weight. The third cohort was administered acyclovir at a dosage of 100 mg/kg of body weight, while the fourth cohort was administered acyclovir at a dosage of 150 mg/kg. The doses were solubilized in aqueous solution and administered to the rodents on a daily basis during the 14-day experimental timeframe.

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

Collection of Blood Samples

Following each treatment, the rats were humanely euthanized through the administration of a lethal dose of chloroform. Cardiac puncture was performed to obtain blood samples from each rat. Blood samples were collected and subsequently transferred to gel test tubes without anticoagulant. The samples were then subjected to centrifugation at a rate of 3000 rpm for a duration of 15 minutes. The resulting serum was utilized for subsequent biochemical analysis. A further aliquot of the blood was obtained and placed into tubes that were supplemented with anticoagulants for the purpose of conducting hematological evaluations, which encompassed the determination of red blood cell (RBC) count, hemoglobin (Hb) concentration, white blood cell (WBC) count, and platelet (PLT) count.

Hematological Tests

All these parameters (RBCs) count (WBCs) count (Hb) concentration and platelet count were measured by count 60 (Gene x laboratories, Germany) apparatus.

Biochemical Measurements

Estimation of Aspartate Aminotransferase (AST), IU/L

"Aspartate aminotransferase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine" [9] according to this equation:

$$\text{X-oxoglutarate} + \text{L-aspartate} \xrightarrow{\text{AST}} \text{L-glutamate} + \text{oxaloacetate.}$$

Estimation of Alanine Aminotransferase (ALT), IU/L

"ALT activity is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenyl hydrazine according to this equation":

$$\text{L-Alanine} + \text{2-oxoglutarate} \xrightarrow{\text{ALT}} \text{pyruvate} + \text{L-Glutamate.}$$

Determination of Alkaline phosphatase (ALP) concentration, IU/L

The estimation is done according to this equation [10]:

$$\text{Phenyl phosphate} \xrightarrow[\text{PH=10}]{\text{ALP}} \text{phenol and phosphate.}$$

Kidney Functions

This was accomplished by the determination of creatinine concentration, which was done according to [11], and the determination of urea concentration [12] in the serum of all experimental rats was treated with acyclovir, compared to the control group.

Semen Examination

Immediately after euthanization, the caudal epididymis of each rat was dissected and placed in a sterile glass Petri dish containing 5 ml of normal saline solution (0.9% NaCl) that had been warmed. The epididymis tissue was finely chopped into small pieces using microsurgical scissors. To avoid any potential cold shock, the tissue was allowed to rest in a water bath at 37 °C for 5 minutes.

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

Epididymis Sperm Concentration

Epididymal sperms were counted according to the methods of [13] by using a Neubauer hemocytometer.

Measurement of Individual Sperm Motility

Individual sperm motility was assessed using a grading system proposed [14]. This system categorizes sperm motility based on the extent of progressive forward movement and the strength and speed of motion. These parameters were then converted into a percentage to determine the level of sperm motility for each sample.

Measurement of Massive Sperm Motility

The extensive mobility of sperm in the epididymis was assessed using the measurement scale established by [13].

Sperms Abnormality

The percentage of abnormal spermatozoa was determined using the same slide that was used to assess the viability of epididymal sperm. A total of 200 sperm cells were counted under a light microscope at a magnification of 100× [13]. The slide was stained with a special eosin-nigrosin stain, which allows for the visualization and identification of abnormal sperm cells. The abnormal spermatozoa were then calculated as a percentage of the total counted sperm cells.

Statistical Analysis

The statistical analysis was performed using SPSS software, specifically version 26. The data collected was presented in the format of mean ± standard deviation. The level of statistical significance was established at $p<0.05$.

■ RESULTS

Effect of Acyclovir on Semen Physiology

The impact of different doses of the acyclovir drug on semen viability varied. The results presented in Table 1 indicated a significant decrease ($p<0.05$) in sperm count, in terms of mass and individual motility (Astenozoospermia), across all groups treated with acyclovir compared to the control group. Additionally, there was a significant increase ($p<0.05$) in sperm abnormality (Teratozoospermia) observed in all acyclovir-administered groups compared to the control group.

Table 1
Effect of Different Doses of Acyclovir on Some Physical Properties of Semen in Adult Male Rats

Parameters Groups	Sperm count ($\times10^6$/ml)	Massive motility (%)	Individual motility (%)	Abnormal Sperm (%)
Control	284.2 ± 0.3^A	97 ± 0.4^A	95.3 ± 0.6^A	3.5 ± 1.1^B
ACV 50 mg/kg	197.2 ± 0.3^B	43.8 ± 0.3^B	37.3 ± 0.3^B	12.8 ± 0.3^B
ACV 100 mg/kg	120.7 ± 1.5^C	28.5 ± 3.5^C	33.5 ± 3.5^C	31 ± 2.6^A
ACV 150 mg/Kg	66.5 ± 2.3^C	13 ± 0.7^C	10 ± 0.6^C	65 ± 1.4^A
LSD	4.5	12.3	7.5	6.8

Note: different letter is significant <0.05.

Effect of Acyclovir on Renal Function

The data presented in Table 2 indicated a significant increase ($p<0.05$) in both urea concentration and creatinine concentration in the acyclovir group with a dosage of 150 mg/kg when compared to the control group and other doses of acyclovir-treated groups. The mean concentration of urea was 32.5±2.6, 37±4.3, and 39.88±5.1, respectively, compared to the control group. Similarly, the mean concentration of creatinine was 1.503±0.216, 1.824±0.112, and 2.203±0.097 in comparison to the control group.

Table 2
Effect of Different Doses of Acyclovir Kidney Function in Adult Male Rats

Parameters / Groups	Urea (mg/dl)	Creatinine (mg/dl)
Control	$30.25±4.1^{C}$	$1.138±3.2^{C}$
ACV 50 mg/kg	$32.5±2.6^{B}$	$1.503±0.216^{B}$
ACV 100 mg/kg	$37±4.3^{B}$	$1.824±0.112^{B}$
ACV 150 mg/kg	$39.88±5.1^{A}$	$2.203±0.097^{A}$
LSD	5.25	0.23

Note: different letter is significant <0.05.

Effect of Acyclovir on Liver enzymes functions

Data and results in Table 3 showed that the concentration of liver enzymes is significantly increased ($p\leq0.05$) in all acyclovir-treated groups at different doses compared with the control group.

Table 3
Effect of different doses of Acyclovir on Liver Enzymes in Adult Male Rats

Parameters / Groups	AST(U/L)	ALT (U/L)	ALP (U/L)
Control	$55.6±1.3^{B}$	$29.4±1.2^{B}$	$113.1±3.6^{B}$
ACV 50 mg/kg	$84.4±1.9^{A}$	$53.8±5.7^{A}$	$137.5±3.4^{A}$
ACV 100 mg/kg	$88.3±6.1^{A}$	$58.4±4.1^{A}$	$176.1±1.6^{A}$
ACV 150 mg/kg	$90.1±2.6^{A}$	$66.3±4.4^{A}$	$180.27±3.2^{A}$
LSD	4.1	5.3	7.4

Note: different letter is significant <0.05.

Effect of Acyclovir on Some Hematological Parameters in Adult Male Rats

The most recent findings presented in Table 4 revealed a "significant decrease ($p\leq0.05$) in hemoglobin levels among rats in both the 100 mg/kg and 150 mg/kg acyclovir-treated groups compared to the control group. Additionally, there was a significant decrease ($p\leq0.05$) in the number of red blood cells and platelets in all treated rats compared to the control group. Moreover, the number of white blood cells exhibited a significant decrease ($p\leq0.05$), specifically in the 150 mg/kg acyclovir-treated rats compared to the other treated and the control groups".

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

Table 4
Effect of Different Doses of Acyclovir on Some Hematological Parameters in Adult Male Rats

Parameters / Groups	Hb, g/dl	RBC, $\times 10^6$/µL	WBC, $\times 10^3$/µL	PLT, $\times 10^6$/µL
Control	13.95±0.86^a	9.01±2.18^b	18.28±1.22^a	797.75±35.83^a
ACV 50 mg/kg	12.85±0.66^{ab}	6.86±0.39^b	18.25±1.83^a	672.75±13.93^b
ACV 100 mg/kg	12.4±0.33^b	6.42±0.29^b	7.06±1.72^b	644.75±20.53^b
ACV 150 mg/kg	12.2±1.06^b	6.61±0.04^b	3.08±0.65^c	629±55.93^b
LSD	1.55	2.15	3.98	125

Note: different letter is significant <0.05.

■ DISCUSSION

Earlier research has indicated that acyclovir possesses efficacy in treating viral infections, but it can also lead to DNA damage in unaffected cells [15]. Several studies conducted by different researchers have reported comparable outcomes of antiviral medications, including Ribavirin and Ganciclovir. These drugs have been linked to a reduction in sperm count and an elevation in sperm abnormality [16, 17]. The current results are aligned with the experimental studies mentioned earlier and support the findings of [7, 18]. These studies indicated that acyclovir treatment decreased overall sperm count and individual and mass motility while also causing increased sperm abnormalities. These results suggest that the activity of acyclovir primarily inhibits viral DNA replication rather than host cell DNA replication, which can potentially impair the functioning of host cells [19]. The current study discovery reveals a noteworthy elevation in both creatinine and urea concentrations among all the groups administered with acyclovir in comparison to the control group. This finding suggests that acyclovir exerts a toxic impact on organs, particularly the kidneys. These results align with the research conducted by [5], who emphasized the nephrotoxic effects of acyclovir in adult male albino rats, where elevated levels of both creatinine and urea nitrogen were observed.

There was a study indicating that the use of acyclovir can potentially result in acute kidney injury (AKI) due to the formation of acyclovir crystals within the renal tubules [20]. While acyclovir is generally well tolerated, there have been instances where severe nephrotoxicity has been reported. AKI induced by acyclovir treatment is characterized by a decline in renal function, typically occurring within 12 to 48 hours after the administration of the drug. This decline is evident through a rapid increase in serum creatinine levels, as highlighted by [21]. Acyclovir-induced nephrotoxicity is commonly observed and can manifest as crystal-induced nephropathy, acute tubulointerstitial nephritis, and rarely as tubular dysfunction. This adverse effect is characterized by increased plasma creatinine levels and abnormal urine findings, as stated by [4]. Furthermore, a recent study revealed a noteworthy reduction in hemoglobin levels, red blood cell count, white blood cell count, and platelet count among rats subjected to acyclovir treatment. These findings align with the research conducted by [22], which emphasized the detrimental effects of antiviral medications, including the development of anemia, thrombocytopenia, and leukopenia.

In addition, the aforementioned results are consistent with the investigation carried out by [23], which demonstrated that the application of favipiravir medication caused a reduction in erythrocyte and leukocyte levels, thereby inducing anemia and thrombocytopenia in healthy rats. Additionally, a previous study by [24] exhibited a

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

noteworthy reduction in erythrocyte count, hemoglobin levels, and hematocrit levels in rats subjected to varying dosages of antiviral medications. The recent findings revealed an elevation in "liver enzyme, namely ALT, AST, and ALP, in all rats treated with acyclovir drugs compared to the control group". This observation may be attributed to the fact that many antiviral agents are utilized to treat liver diseases, and most drugs' metabolism occurs within the liver. As a result, mild to moderate side effects may arise from the use of these drugs.

These results are aligned with a previous study suggesting that some hepatotoxic effects associated with antiviral agents stem from the altered metabolism of other drugs, with the liver and its specific enzymes playing a crucial role in this process [25]. Additionally, a separate investigation reported a noteworthy increase in the hepatic enzyme levels, specifically AST, ALT, and ALP, among rabbits administered with acyclovir therapy compared to those without treatment. Furthermore, a significant elevation in uric acid levels was observed in the group that received treatment. Nevertheless, the treated and control groups did not exhibit noteworthy disparities in urea and creatinine levels [26].

■ CONCLUSION

Astenozoospermia and teratozoospermia are commonest seen in semen analysis after acyclovir consumption. A significant decrease of sperm count and elevated of sperm abnormality are the major adverse effect of acyclovir. Raising of renal function is an indicator of renal failure due to acyclovir. Liver enzymes elevation is a sign of toxicity of acyclovir. The presence of thrombocytopenia, leucopenia, a reduced count of red blood cells and platelets, and a decline in hemoglobin levels in all the rats treated with acyclovir.

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The Effect of Methotrexate on Oogenesis of Laboratory Female Mice

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Abstract

Introduction. This investigation was carried out by studying the histological sections of the ovaries of female mice which are treated with methotrexate and its effect on the sex hormone estrogen.
Purpose. The current study aimed to investigate the extent to which methotrexate (MTX) concentrations affect the oogenesis process and influence the ovary in general and the ovarian follicles in particular.
Materials and methods. In this study, 24 female laboratory mice (Mus musculus L.) were used Female laboratory mice were divided into three groups, each containing eight mice. Control female mice were injected with 0.1 ml of distilled water into the intraperitoneal region, the first treatment group of female laboratory mice was injected with methotrexate at a concentration of (2 mg/kg), and the second treatment group was also injected with the drug at a concentration of (4 mg/kg) daily for 16 days.
Results. The results of examining the histological sections of the ovaries of female laboratory mice which are treated with methotrexate showed clear histological changes in both treatment groups. It was found that the ovaries of females that were treated with the low concentration appeared less affected histologically than the high concentration, and the number of ovarian follicles was similar to that in the control group. The results also showed a significant decrease in the concentration of estrogen hormone for both groups treated with the drug (2.4 mg/kg) when compared to the concentration of the hormone in the control group of mice at a probability level of $P<0.05$.
Conclusion. The present study concluded that methotrexate affected the oogenesis process through affecting the ovaries directly, causing histological changes in the ovarian follicles. Further, methotrexate affected indirectly the estrogen hormone, causing a decrease in the levels in treated mice, which in turn affects the oogenesis process. Furthermore, the percentage of ovarian damage in mice which are treated with a high dose of methotrexate was greater histologically than that in mice which are treated with a low dose. Accordingly, the decrease in fertility of treated female mice increased with increasing dose.
Keywords: methotrexate, estrogen, ovary, oogenesis, histological changes

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

INTRODUCTION

The oogenesis process, like other vital processes in the body, is affected by the side effects of chemotherapy which is used to treat many diseases. One of these chemotherapy treatments is methotrexate which is used to treat many inflammatory and autoimmune diseases, especially rheumatoid arthritis, as well as cancerous tumors [1]. Many side effects of this drug have been observed on the male and female reproductive system, leading to infertility in both sexes [2].

One of the most important side effects of using anti-cancer drugs is the loss of ovarian function, which may result in infertility or decreased fertility rates, especially when using these drugs in the long term [3, 4]. In addition, anti-cancer treatments negatively affect the body's functions, causing a decrease in the production of sex hormones, menopause in women, and sexual dysfunction [5].

However, methotrexate toxicity has been linked to female fertility through its effect on follicle number, egg reserve, and ovarian response, besides its effect on follicle-stimulating hormone (FSH) and luteinizing hormone (LH) [6, 7]. Moreover, McLaren et al., 2009 [8] showed the effect of treating female hamsters with methotrexate on egg production, which in turn caused a disturbance in the fertility level through the oocytes' sensitization to the drug's toxicity [9].

The current study aims to investigate to what extent methotrexate concentrations affect the oogenesis process and influences the ovary in general and the ovarian follicles in particular. This investigation was carried out by studying the histological sections of the ovaries of female mice that are treated with methotrexate and its effect on the sex hormone estrogen.

MATERIALS AND METHODS

Laboratory Mice

The current study used female laboratory mice (Mus musculus L.) aged 12 to 14 weeks and weighing 20–22 g. Laboratory mice were adapted to the optimal conditions of temperature (25±2 °C) and standard food. The experiments were conducted in the animal house of the Department of Biology / University of Basrah after being raised and fed [10, 11].

Experiment Design

The twenty-four female laboratory mice were divided into three groups, each containing eight mice. The control female mice were injected with 0.1 ml of distilled water in the intraperitoneal region, while the first treatment group of laboratory female mice was injected with methotrexate provided by (KOCAK FARMA TURKY) at a concentration of 2 mg/kg daily for 16 days. The second treatment group was also injected with methotrexate at a concentration of 4 mg/kg daily for 16 days as well.

Hormonal Assessment

After 16 days of being treated with methotrexate, the mice were anesthetized with chloroform, blood was drawn directly from the heart, and the serum was separated and placed in special tubes for preservation. Using the ELISA method and the competitive interaction principle, the concentration of estrogen hormone was measured in the serum

of mice at a wavelength of 450 nanometers, using the measurement kit provided by the German company "Human". The hormone concentration values were subjected to statistical analysis using SPSS and One Way ANOVA analysis at a probability level of less than 0.05.

Histological Preparation

For Preparation of tissue sections, laboratory mice ovary samples were fixed in 10% formalin for 24 h, and then the samples were washed with water and preserved in 70% ethyl alcohol. The samples were passed through a series of ascending alcohol concentrations of 90% and 100%. They were cleaned with xylene and the ovaries were soaked in molten paraffin wax, changing the wax every 30 mins for three times. The samples were embedded using special molds, cut to a thickness of 7 mm, and placed on glass slides. They were stained with hematoxylin and eosin, and then they were examined and photographed with a Leica microscope [12].

■ RESULTS

1. The Effect of Methotrexate on the Oogenesis Process in the Ovaries of Female Laboratory Mice

The results of examining the histological sections of the ovaries of female laboratory mice treated with methotrexate showed clear histological changes at both low and high concentrations. The ovaries of females treated with low concentration appeared less affected histologically than that of high concentration and the number of ovarian follicles was similar to that in the control group (Fig. 1A and B).

The study of the histological sections of the low concentration group showed degenerative changes from which the ovarian follicles suffer, whether in the oocyte or the follicular cells of the surrounding granulosa membrane. The degenerative changes of the oocyte were represented by shrinkage and withdrawal of the Ooplasma to one side, the absence of the nucleus and nucleolus, and the absence of the zona pellucid (Fig. 1C). In some ovarian follicles, a change in the shape of the nucleus of the oocyte was observed, as the nuclear envelope appeared irregular (Fig. 1D). In addition, the absence of the oocyte with the preservation of the ring-shaped nucleus containing a small, lateral-located nucleolus (Fig. 2A), and the degrade of the oocyte (Fig. 2B and C) were also observed. Moreover, the various changes that the follicular cells of the granular membrane of the ovarian follicles went through were very clear. Among these changes were the irregularity of the follicular cells and their degeneration (Fig. 2B), and the occurrence of apoptosis, which led to the appearance of spaces, whether between the follicular cells within the granular membrane or between the oocyte and the surrounding follicular cells (Fig. 1C and D, Fig. 2C and D).

As regards the ovaries of the high concentration group, the histological sections showed a reduction in the number of ovarian follicles and that most of these follicles suffered from degenerative changes, with the presence of bleeding in different places in the ovary. In some other histological sections, clear degenerative changes were observed in the medullary region of the ovary, appearing in the form of vacuoles (Fig. 3A and B).

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

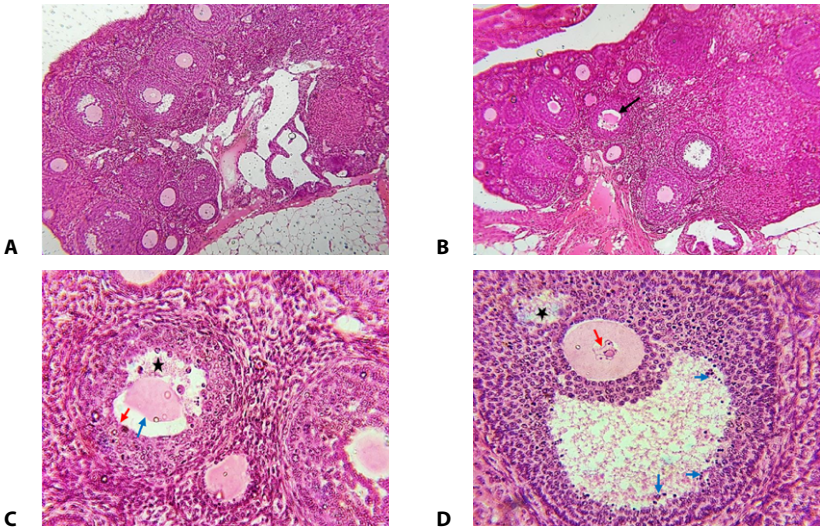

Fig. 1. A – Cross section in mice ovary of control group showing ovarian follicles in different growth stages 100×; B – Cross section in mice ovary of treatment group (low dose), notice number of ovarian follicles, similar to number of ovarian follicles in control group. Degenerative changes in ovarian follicle (arrow) 100×; C – ovarian follicle of treatment group (low dose), notice shrinkage of Ooplasma to on side (blue arrow), absence of zona pellucida (red arrow) and absence of nucleus and nucleolus, with occurrence degenerative changes in some follicular cells near ovum (stare) 400×; D – ovarian follicle of treatment group (low dose), notice an irregular nucleus envelop (red arrow),with occurrence of degenerative changes in membrane granulosa(stare) and apoptosis in some follicular cells near atrium (blue arrows) 400×

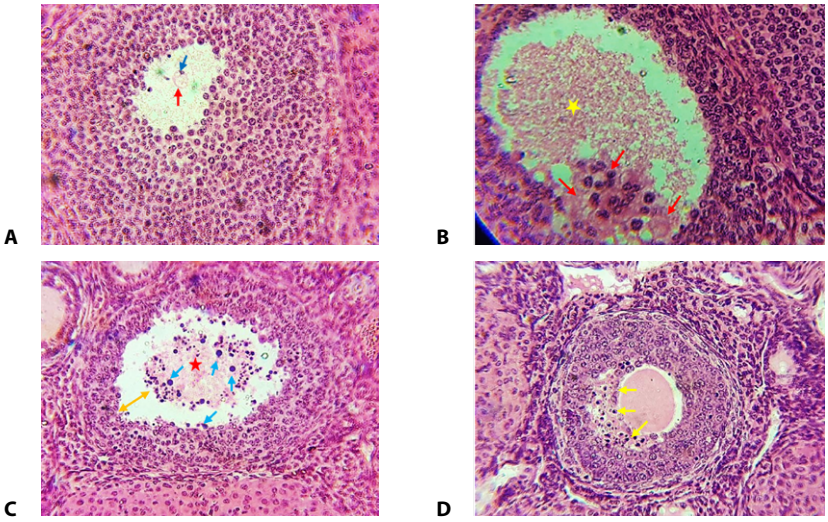

Fig. 2. A – ovarian follicle of treatment group (low dose), notice the nucleus in ring form (red arrow) and the nucleolus located in one side of nucleus (blue arrow) 400×; B – ovarian follicle of treatment group (low dose), notice ovum cytolysis (stare), irregular and degenerated follicular cells (red arrows) 400×; C – ovarian follicle of treatment group (low dose), notice ovum cytolysis (stare), apoptosis in follicular cells (blue arrows) and the space between ovum and follicular cells of membrane granulosa (two head arrow) 400×; D – ovarian follicle of treatment group (low dose), notice the occurrence of degenerative change in follicular cells near one side of ovum (yellow arrows) 400×

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

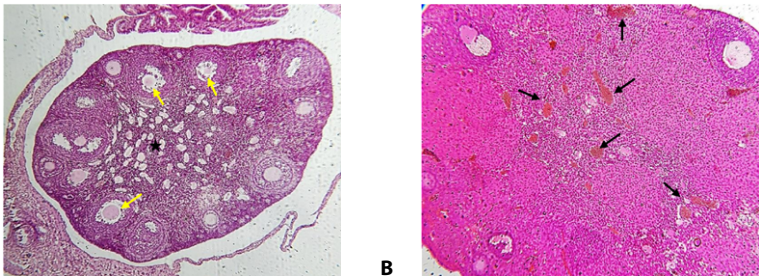

Fig. 3. A – cross section in mice ovary of treatment group (high dose), notice degenerative changes in medulla of ovary in vacuoles form (stare) and in ovarian follicles (yellow arrow) 100×; B – cross section in mice ovary of treatment group (high dose), notice the occurrence of bleeding (arrows) and reduction number of ovarian follicles 400×

2. The Effect of Methotrexate on the Concentration of Estrogen in the Serum of Female Laboratory Mice

When statistically evaluated, the treatment of female mice with methotrexate chemotherapy for 16 days caused a significant decrease in the concentration of estrogen hormone for both groups treated with the drug (2.4 mg/kg) when compared to the concentration of the hormone in the control group of mice at the probability level of $P<0.05$ (Fig. 4).

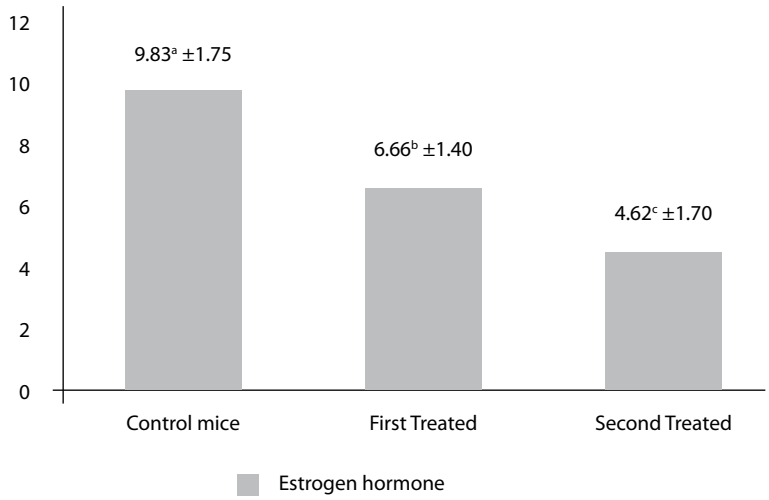

Fig. 4. Effect of methotrexate on estrogen hormone concentration of mice

Notes: [a, b, c] Difference of lowercase letters indicates significance at the $P<0.05$.

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

DISCUSSION

Methotrexate (MTX) is a type of chemotherapy which is used to treat many inflammatory diseases, autoimmune diseases, and cancer [1]. Despite its therapeutic importance, it was found that its effect is not only limited to treating cells affected by these diseases, but also it affects normal, rapidly proliferating cells, especially reproductive system cells [13]. MTX has a side effect on the processes of oogenesis and spermatogenesis [2], by targeting reproductive cells, including oocytes and granulosa cells, which leads to a reduction in the oocyte reserve in the ovary [14].

The results of examining the histological sections of the ovaries of female laboratory mice which are treated with methotrexate showed clear histological changes at both low and high concentrations. The ovaries of females treated with the low concentration appeared less affected histologically than that of the high concentration, and the number of ovarian follicles was similar to that in the control group. The degenerative changes in the oocyte were represented by shrinkage and withdrawal of the ooplasma to one side, the absence of the nucleus and nucleolus, and the absence of the zona pellucid. It was also noticed that there was a change in the shape of the oocyte nucleus in some ovarian follicles, as the nuclear envelope appears irregular.

The results of the current study were consistent with the results of the studies conducted by Chelab and Majeed, 2009 [15] and Battan et al., 2016 [16]. The results of those studies showed degenerative changes in the ovarian follicles in the ovaries of female laboratory mice after injecting them with MTX, as well as a significant decrease in the number of Graafian follicles and the absence of the corpus luteum or its weak growth. Exposure to methotrexate causes apoptosis of follicular granulosa cells and abnormalities in the development of ovarian follicles [17]. This is because MTX is one of the common folic acid antagonists, as it prevents or competes with the reduction of dihydrofolate resulting from folic acid metabolism to tetrahydrofolate, which is the active form of folic acid. It also plays a role in some metabolic products necessary for DNA formation and modifies the methylation process of DNA. Thus, it affects the inhibition of DNA formation and damage, which causes cell proliferation disorder and apoptosis [18]. In addition, it inhibits the growth and maturation of ovarian follicles in the ovaries [19, 20].

This explained the occurrence of degenerative changes in either the oocyte or the ovarian follicles in the ovaries of female mice treated with methotrexate in the current study. Also, the shrinkage and withdrawal of the ooplasma to one side resulted from the increased programmed death processes under the influence of methotrexate of the granulosa cells and their loss, which led to a defect in the connection of those cells with the ooplasma of the oocyte and then its shrinkage [21].

The occurrence of histological changes in the ovaries of female mice treated with methotrexate can also be attributed to increased oxidative stress, inactivation of antioxidants, increased production of many proinflammatory mediators, and, consequently, increased programmed cell death of ovarian cells [22]. It was observed that there was an increase in the production of Caspase-2,3 enzymes in the ovaries of females treated with methotrexate, which are two important mediators of apoptosis and, consequently, damage to the tissues of the ovaries of the treated females [23].

Histological sections of ovaries treated with high concentration of the drug showed a reduction in the number of ovarian follicles and that most of these follicles suffered from degenerative changes, with bleeding in different places in the ovary. It was also noticed

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

that in some other histological sections, clear degenerative changes occurred in the medullary region of the ovary, taking the form of vacuoles. The results of the current study were consistent with the results of Gol et al., 2009 [24], who stated that the use of a high dose of the drug (25 g/m) caused tissue damage to the primary follicles and a decrease in their numbers, as well as the destruction of the ovarian follicles of the ovaries of female mice treated with methotrexate.

The reason for these degenerative tissue changes occurred in the high dose may be due to the increased accumulation of reactive oxygen species (ROS) resulting from the use of the drug, which leads to an increase in oxidative stress in the ovarian tissues. The increase in ROS inhibits cytoplasmic and nuclear maturation. Thus, the occurrence of apoptosis, or necrosis may happen in the ovarian tissues as a mechanism or reaction to the damage caused by the use of this chemotherapy [25, 26].

Moreover, chemotherapy treatments, including methotrexate, cause genotoxicity to germ cells in mice, using high doses of 5, 10, 20, 40 mg/kg [27]. Exposure to MTX also leads to an imbalance in nucleotides and the incorporation of the nitrogenous base uracil into DNA, thus leading to cell death resulting from toxic stress [28].

Sex hormones play an important role in the process of growth and maturation of ovarian follicles. A disruption in the production of these hormones negatively affects the oogenesis process. The results of the current study of female laboratory mice treated with methotrexate showed a significant decrease in the concentration of estrogen hormone for both groups treated with the drug and the two doses (2,4 mg/kg) when compared to the concentration of the hormone in the control group. This deficiency in sex hormones such as the estrogen may be attributed to severe damage to the ovarian follicles caused by the use of methotrexate chemotherapy [29].

The results of the current study were consistent with the results of the studies conducted by Manna et al., 2003 [30] and Karri & Vanithakumari, 2011 [31], which showed that exposure to methotrexate inhibits the formation of steroidogenesis and the ovarian functions through its effect on reducing the levels of gene expression of the steroidogenic acute regulatory protein (StAR), which in turn stimulates the ovarian follicles to produce sex hormones. It is also probable that the drug affects the control system of the pituitary gland-hypothalamus and the uterus, ovaries and oviducts, causing disturbances in the reproductive process [32].

It is evident that the production of estrogen inhibits apoptosis of granulosa cells and assists the ovarian follicles to mature [33], and that its decrease increases programmed cell death of granulosa cells. Therefore, the decrease in estrogen concentration in the current study has a significant effect on the degenerative changes of ovarian follicles that occurred in the ovaries of female mice under study.

■ CONCLUSION

The current study concluded that MTX affects the oogenesis process. MTX has a direct toxic effect on the ovaries, causing histological changes in the ovarian follicles. Indirectly, it affects the estrogen hormone and its decrease in its levels in the treated mice, which in turn affects the oogenesis process. The percentage of ovaries of mice treated with a high dose of the drug was more affected histologically than that of the ovaries of mice treated with a low dose. Thus, the decrease in fertility of female mice treated with MTX increased with increasing dose.

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The Ameliorated Effect of Hesperidin on Testicular Disorders in Male Rats Treated with Cyclophosphamide Chemotherapy

Conflict of interest: nothing to declare.
Authors' contribution: Ghadeer A. Jabbar – conceptualization, data curation, investigation, methodology, project administration, software, validation, visualization, writing – original draft and writing – review & editing; Faris S. Kata – conceptualization, data curation, project administration, resources, supervision, visualization, writing – original draft and writing – review & editing.
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Abstract

Introduction. Cyclophosphamide is one of the most widely used chemotherapy treatments. It is an alkylating agent belonging to the Oxazaphosphorine class.
Purpose. The current study aimed to investigate how hesperidin can prevent the damage caused by cyclophosphamide. It also looked at its impact on sperm count, the frequency of sperm abnormalities, histological changes in testis and testosterone levels in rats.
Materials and methods. Thirty-two adult males were involved in the research divided randomly into four groups of eight rats each. The control group received a 1 ml dose of a normal saline while the treated group was injected with 1 ml of cyclophosphamide at a concentration of 12 mg/kg, in the intraperitoneal region. The group treated with the low dose was given an oral gavage of 1 ml of hesperidin solution at a concentration of 15 mg/kg one hour after injection with cyclophosphamide. The group treated with the high dose was injected a dose of 1 ml of hesperidin at a concentration of 30 mg/kg one hour after the injection with cyclophosphamide.
Results. A significant decline in the epididymal sperm count. In addition to a discernible drop in Testosterone levels in blood serum, the incidence of malformations rose. On the histopathological side, cyclophosphamide injection resulted in Sertoli cell degeneration, germ cell irregularities lining the seminiferous tubules, and the inhibition of spermatogenesis in the testes.
Conclusion. Hesperidin improved outcomes for both groups by increasing sperm count, decreasing deformity rate, raising serum testosterone concentration, and improving testes histologically by reducing cellular degeneration and revealing the sequence of germ cells, just like in a normal situation. Furthermore, it enhances the quantity of sperm inside the seminiferous tubule lumen. Despite the damaging effects of chemotherapy on the testes, hesperidin protected and improved male laboratory rats' fertility.
Keywords: cyclophosphamide, hesperidin, spermatogenesis, testosterone, testes, male rats

INTRODUCTION

Cyclophosphamide is one of the most widely used chemotherapy treatments. It is an alkylating agent belonging to the Oxazaphosphorine class. Its chemical formula is C7H15C12N2O2P [1]. It was first synthesized in 1958 by Bourseaux and Brock. The active metabolites of cyclophosphamide, Phosphoramid Mustard, and Acrolein are responsible for the cytotoxic side effects [2]. Cyclophosphamide is used to treat types of cancers, such as Acute and Chronic Leukemia, Lymphomas, Ovarian Carcinoma, Neuroblastomas, Sarcoma, and autoimmune tumors [3]. There are many harmful side effects associated with cyclophosphamide in humans and experimental animals, such as anemia, hemorrhagic cystitis, diarrhea, hair loss, and gonadotropin toxicity [4]. Nitrogen Mustard drugs, including cyclophosphamide, exert their biological activity through DNA alkylation, i.e., binding to DNA and separating its strands. This prevents DNA replication and cell death. It not only prevents the growth of cancer cells but also prevents the growth of all rapidly growing cells in the body [5].

After the hydroxylation process of cyclophosphamide by the Cytochrome P-450 enzyme in the liver, the toxic compound Phosphoramide Mustard (PAM) is produced, which is a compound that causes cell toxicity by cleaving DNA. Acrolein is responsible for producing ROS that causes tissue injury [6]. The alkylating effects of the treatment affect the process of sperm formation, which leads to infertility, which appears in the form of testicular atrophy, reduced motility and number of sperm, and increased deformities. It also affects the level of sex hormones [7, 8].

On the other hand, there are natural substances that have a protective effect against toxins caused by chemical treatments in the body, including antioxidants, which are defined as a group of elements that can prevent or slow down the oxidation process that produces molecules called free radicals. Thus, it protects the body from oxidative stress [9,10]. Among these antioxidants is hesperidin, which belongs to the flavanone compounds, which are one of the subgroups of flavonoids [11]. They work to improve fertility by increasing the production of sperm numbers and motility and protecting them from ROS. It also reduces oxidative damage that causes toxicity to the system. Male reproductive function, alleviating reproductive defects, and improving testosterone levels in laboratory rats [12].

The study aims to evaluate the preventive and improving effect of hesperidin on the physiological and biochemical effects and changes induced by cyclophosphamide in laboratory rats.

MATERIALS AND METHODS

Laboratory animals

In the research we employed Wister rats weighing, between 200 to 250 grams in our laboratory. These rats were given a week to adjust to the controlled environment, which maintained a temperature range of 20 25°C and followed a lighting schedule of 12 hours light and 12 hours dark. The rats were provided with access, to both water and food utilizing forage as their source of sustenance.

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

Experiment design

Male sexually mature laboratory rats at three months of age were divided into four equal groups, each consisting of 8 rats, as follows:

1. Control group: injected into the intraperitoneal (I.P.) and dosed with 1 ml of normal saline.
2. The group treated with cyclophosphamide: injected with a volume of 1 ml of cyclophosphamide solution at a concentration of 12 mg/kg.
3. The low-dose treatment group: A dose of 1 ml of hesperidin solution at a concentration of 15 mg/kg was administered one hour after being injected with a cyclophosphamide solution at a concentration of 12 mg/kg.
4. The high-dose treatment group: A dose of 1 ml of hesperidin converter at a concentration of 30 mg/kg was administered one hour after being injected with a cyclophosphamide solution at a concentration of 12 mg/kg.

Method and duration of injection

Laboratory rats were injected daily for 21 days into the intraperitoneal (I.P.) at a concentration of 12 mg/kg based on [13]. As for the two treatment groups, they were dosed with Oral gavage at a concentration of 15 and 30 mg/kg one hour after the injection, based on [14].

Preparation of blood serum

Drawing blood from the heart immediately the day after the last injection. The animals were fasting by anesthetizing the animals with chloroform, then withdrawing the serum separated from the clotted blood and storing it in Eppendorf tubes at a temperature of 10°C until the hormonal examination was performed [15].

Laboratory experiments

Physiological tests

The method adopted by [16] in calculating the number of sperm by taking the right epididymis from the rat testes and cutting it with a blade into very small pieces in a glass Petri dish containing 1 ml of physiological buffer solution. Then, 1–3 drops of Eosin stain were added to it and kept for 10 minutes to stain sperm. The solution containing the sperm is then withdrawn using a white blood cell pipette to the mark of 0.5 and diluted to the mark of 11 using the physiological solution. Then, a drop of it is added to the hemocytometer slide. Then, the numbers of sperm in the squares for white blood cells are counted. The total number of sperm (N) is multiplied according to the following equation:

Total number of sperm in the epididymis = $10^3 \times 50 \times N$

For the purpose of studying sperm abnormalities, the method of [17] was adopted. The epididymis was extracted and cut into very small pieces in a Petri dish containing 10 ml of physiological buffer solution. Then, the resulting solution is put in a test tube. Using a clean dropper, drop the sperm solution at an appropriate height onto 5 adjacent glass slides so that the solution can spread with the sperm it contains. Then, leave 5 minutes to dry. The slices are then placed in a Coplin jar containing eosin stain for 5-10 minutes. The excess stain is then removed with distilled water and left to dry until it is ready for examination with an optical microscope under 40× magnification, with 100 sperm per slide. Finally, a comparison was made with the natural shapes of sperm in the control group.

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

Biochemical tests

The current study included biochemical tests detected in the serum of laboratory rats. It included testing the level of the sex hormone Testosterone. The test kit prepared by Elabscience was used. The hormone level was measured according to the competitive reaction at a wavelength of 450 nm using an ELISA reader.

Histological study

Testicle samples were taken immediately after autopsy and preserved in formalin (10%) for 24 hours. Then, it was washed with water for 24 hours and stored in ethyl alcohol (70%) until the samples were used. The samples were then passed through different concentrations of alcohol to remove water from them. The samples were then soaked in xylene. Then, the samples were impregnated in melted paraffin wax in the oven. It was then buried in special molds and left to harden until the cutting process was carried out. The samples were then cut at a thickness of 7 micrometers using a Rotary microtome device and stained using hematoxylin and eosin. The tissue sections were loaded with Canada Balsam and covered with a clean slide cover. They were left to dry at room temperature, and the tissues were examined under an imaging microscope [18].

Statistical analysis

The statistical program (SPSS) version 21 Statistical Package for the Social Sciences was used to analyze the results statistically using the ONE-WAY ANOVA test. The least significant difference test was used at the $P<0.05$ level.

■ RESULTS

Table 1 shows a significant decrease in the total number of sperm after treatment with cyclophosphamide at a dose of 12 mg/kg at a probability level of $P<0.05$ when compared with the control group. On the other hand, treatment of rats with hesperidin caused a significant increase in sperm counts compared to the group treated with cyclophosphamide, and no significant difference appeared between the treatment group and the control group.

Table 1
The effect of cyclophosphamide on sperm count and the improving role of hesperidin in laboratory rats (n=8) (mean±SD)

Parameter	Total number of sperm $\times 10^6$
Control group Physiological solution 1 ml	226.84[a] ±27.84
Group treated with cyclophosphamide 12 mg/kg	64.18[b] ±11.68
Low dose group (cyclophosphamide 12 mg/kg with hesperidin 15 mg/kg)	213.45[a] ±31.6
High-dose treatment group (Cyclophosphamide 12 mg/kg with Hesperidin 30 mg/kg)	217.27[a] ±55.01

Note: the contrast of lowercase letters indicates significant differences.

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

Table 2 shows that there is a significant increase in the number of deformed sperm after treatment with cyclophosphamide at a dose of 12 mg/kg at the level P<0.05 when compared with the control group. In addition, hesperidin caused a significant decrease in the percentage of deformities in the groups treated with hesperidin compared with the control group.

The effect of cyclophosphamide on sperm abnormalities and the improving role of hesperidin in laboratory rats (n=8) (mean±SD) (Table 2).

Table 2
The effect of cyclophosphamide on sperm abnormalities and the improving role of hesperidin in laboratory rats (n=8) (mean±SD)

Parameter	Normal sperm	Deformed sperm %
Control group Physiological solution 1 ml	80.00[a] ±4.11	20.10[c] ±4.11
Group treated with cyclophosphamide 12 mg/kg	32.56[c] ±7.86	67.43[a] ±7.86
Low dose group (cyclophosphamide 12 mg/kg with hesperidin 15 mg/kg)	68.50[b] ±2.35	31.50[b] ±2.35
High-dose treatment group (Cyclophosphamide 12 mg/kg with Hesperidin 30 mg/kg)	65.00[b] ±2.21	35.00[b] ±2.21

Note: the contrast of lowercase letters indicates significant differences.

Injection of cyclophosphamide into laboratory rats resulted in a significant decrease in the level of Testosterone compared to the control group. In contrast, treatment of rats with hesperidin at both doses caused a clear and significant improvement compared to the dose treated with the drug, as its concentration did not change with the control group and at the probability level P<0.05 (Table 3).

Table 3
The effect of cyclophosphamide on testosterone levels and the improving role of hesperidin (n=8) (mean±SD)

Parameter	Testosterone ng/mL
Control group Physiological solution 1 ml	3.65[a] ±0.85
Group treated with cyclophosphamide 12 mg/kg	0.84[b] ±0.09
Low dose group (cyclophosphamide 12 mg/kg with hesperidin 15 mg/kg)	4.03[a] ±1.57
High-dose treatment group (Cyclophosphamide 12 mg/kg with Hesperidin 30 mg/kg)	3.82[a] ±0.98

Note: the contrast of lowercase letters indicates significant differences.

Histological effects of cyclophosphamide in testes and the Ameliorating role of hesperidin

Histological sections of the testes of the control group showed the observation of all types of germ cells in the seminiferous tubule, sperm progenitors, primary sperm cells, spermatids, and sperm, with the presence of Leydig cells between the seminiferous

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

tubules. The natural arrangement of the types of germ cells shows the sequential stages of spermatogenesis, the interspaces between the seminal tubules containing Leydig cells, and the seminal tubules' sizes are equal, Figure (A). Histological sections of the group treated with cyclophosphamide also showed vacuolar degeneration in most of the seminiferous tubules. It was noted that the germ cells were irregular, and the sperm formation process was absent. This means that some seminal tubules contain only sperm precursors and primary spermatocyte, and the number of sperm in the lumen

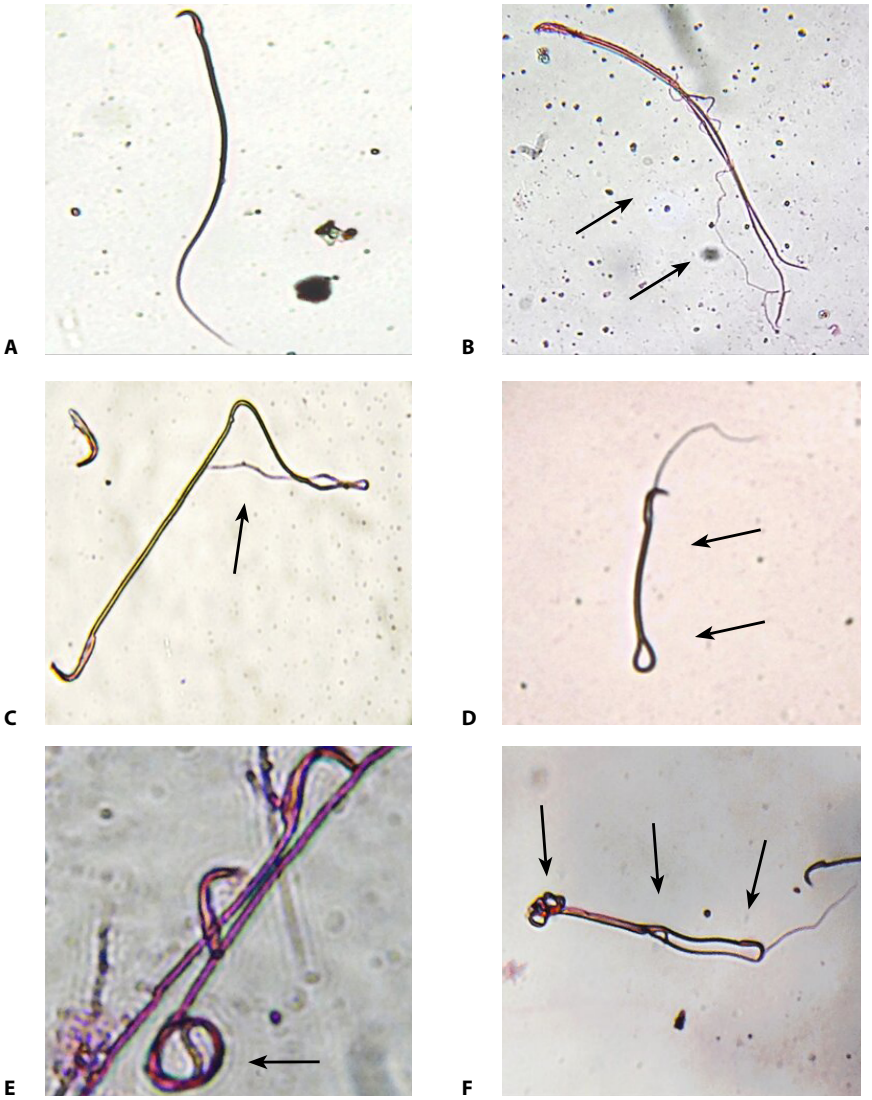

Fig. 1. Sperm abnormalities: A – Normal sperm; B – Cleavage of the middle and tail of the sperm; C – Wrapping of the sperm tail; D – Fusion of the tail with the central region and the toroidal end; E – Sperm with an 8-shaped end; F – Adhesion of a sperm group

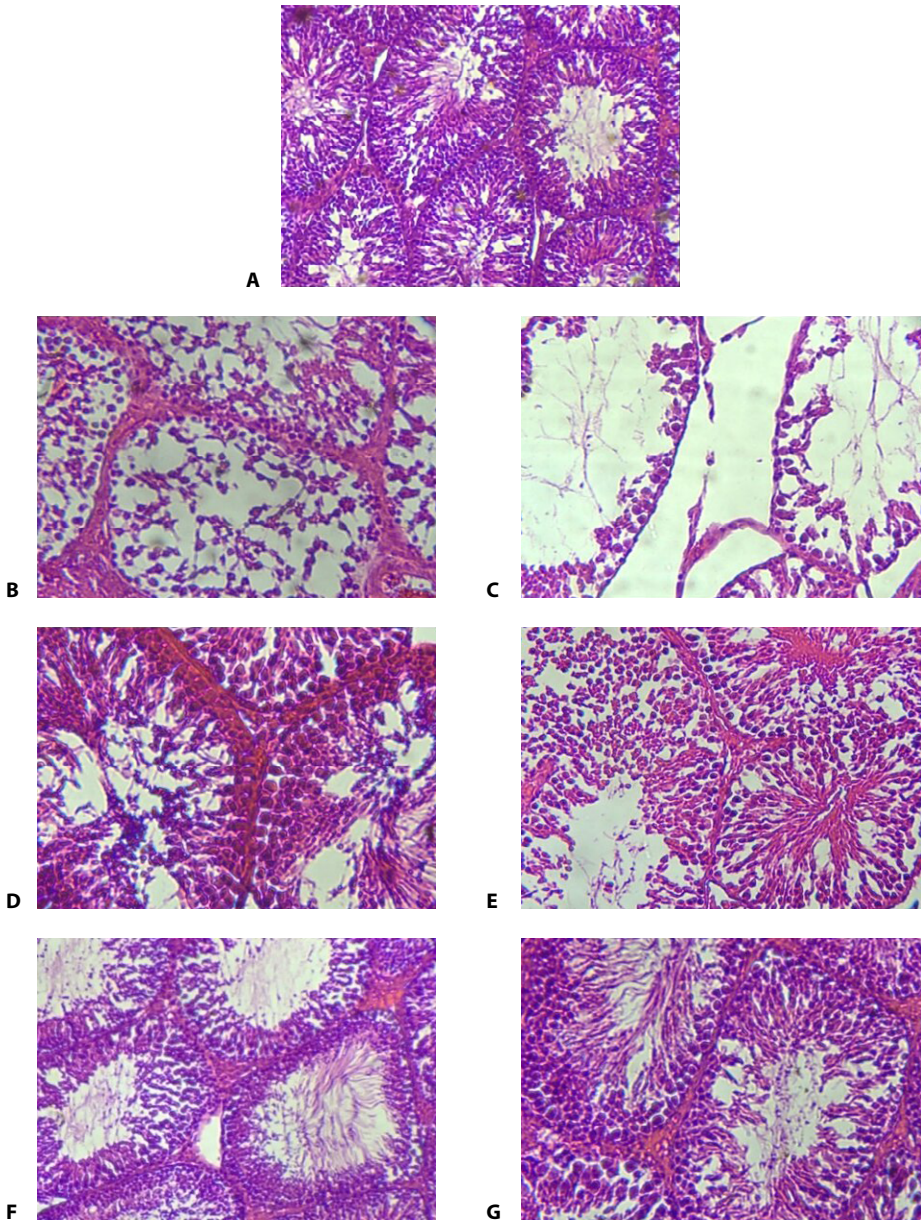

Fig. 2. Cross-sections of testicles of laboratory rats, H&E, ×100: A – Cross-section of testicles of the control group; B–C – A cross-section of the testes of rats treated with cyclophosphamide showing the lack of sperm in the lumen of the seminiferous tubule, the large interspaces between the seminiferous tubules, the sloughing of germ cells in the lumen of the seminiferous tubule, and the presence of vacuolar degeneration in Sertoli cells; D–E – A cross-section of the testicles of a group of rats treated with a low dose of hesperidin showing the reduced number of cellular degeneration in Sertoli cells, the irregularity of the germ cells of the seminiferous tubule, and the regularity of the seminiferous tubules; F–G – A cross-section of the testes of a group of rats treated with a high dose of hesperidin showing the process of spermatogenesis and the organization of germ cells and Leydig cells in their normal form

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

of some seminal tubules is low compared to the control group. It was also observed that vacuolar degeneration occurred in Sertoli cells, and the interspaces between the seminiferous tubules grew due to irregularity in the size of the seminiferous tubules. Gaps also appeared between the Leydig cell clusters and the sloughing of germ cells in the lumen of the seminiferous tubule, images (B-C). In addition, as was observed in the group treated with the low dose of hesperidin, there was regularity in the size of the seminiferous tubules, the absence of large interspaces between the seminiferous tubules, the presence of Leydig cells in their normal form between the seminiferous tubules, and the presence of spermatogenesis through the presence of a sequence of germ cells, as is the case in the case. Natural In addition to an increase in the number of sperm present in the lumen of the seminiferous tubule compared to the treated group and the presence of degenerative gaps in the germinal tissue lining some seminal tubules, which are less than in the treated group (images (D–E). As for the group treated with the high dose, the seminal tubules were more like their normal state. The seminal tubules appear regular in shape, and the space between them contains Leydig cells in their natural shape. The germ cells are organized in the seminiferous tubule. Observing the sequence of the process of sperm formation through the presence of most of the germ cells, spermatogonia, the primary spermatocyte, spermatids, and the sperm is similar to the normal condition (F–G).

■ DISCUSSION

The results of the current study showed a significant decrease in sperm counts after treating male laboratory rats with cyclophosphamide when compared with the control group, which agreed with the study [19]. In general, cytotoxic drugs suppress the spermatogenesis in laboratory animals due to damage to germ cells through the cessation of their division and their loss through apoptosis. It results in a reduction in the rate of sperm produced [20]. The toxicity of cyclophosphamide appears through an increase in ROS that generates oxidative stress, which causes significant damage to the process of spermatogenesis, which includes a decrease in the number of germ cells, especially spermatogonia cells and primary spermatocytes, which are more affected by the drug [21]. The decrease in sperm numbers may also be due to the drug cyclophosphamide causing severe damage to Sertoli cells. These cells play a role, in supporting the growth, differentiation and nourishment of spermatogenic cells as they mature into sperm. They also contribute to the sloughing of germ cells into the seminal tubule lumen resulting in a decrease in sperm count [22]. The decline in sperm count may be linked to reduced levels of Testosterone. In a research involving rats treated with cyclophosphamide, a drug that plays a role in sperm development and maturation this outcome was validated [23]. Testosterone hormone is a key for sperm production as it prompts sperms to multiply. Any disruption in its levels in the blood serum can have effects on sperm production. Thus, the decline in hormone levels observed in our study coincided with a decrease, in sperm count [24].

Cyclophosphamide also led to a reduction, in follicle stimulating hormone (FSH) which plays a role in the development of Sertoli cells. These cells are responsible for supporting the process of sperm formation and maturation producing a nurturing fluid for sperm transforming spermatids into sperm and generating androgen binding protein. This protein serves as a receptor for Testosterone on Sertoli cells highlighting the significance of maintaining a level of Testosterone, for optimal sperm development [25].

On the other hand, the current study confirmed the preventive role played by hesperidin in alleviating the harmful effects on sperm and reproductive system toxicity caused by cyclophosphamide by increasing sperm numbers by protecting against oxidative stress and damage caused by free radicals. The results of the current study agreed with the study of [26]. Flavonoids have an inhibitory effect on lipid peroxidation, thus reducing MDA concentration and increasing the ability and effectiveness of antioxidants, thus increasing sperm numbers [27]. In addition, antioxidants play an important role in protecting the testicles from oxidative stress and tissue damage. Flavonoids suppress the formation of ROS either by activating antioxidant enzymes or by chelating trace elements involved in the generation of free radicals. Hesperidin also caused inhibition or suppression in the expression of the p53 gene in testicular tissue, which led to a decrease in cell death in the testicular cells responsible for sperm formation [28].

The results of the current study showed a significant increase in deformed sperm in animals treated with cyclophosphamide compared to the control group, and the result was consistent with the result [19]. Sperm are more susceptible to oxidative damage than other cells due to the high concentration of polyunsaturated fatty acids in their membranes and the low concentrations of antioxidant enzymes in their cytoplasm. Increased ROS and oxidative stress due to acrolein attack unsaturated fat bonds and thus oxidize fatty acids. Peroxides, alcohols, and fatty aldehydes are produced as byproducts of the reaction. Fat peroxidation destroys the structural structure of fats in sperm membranes and increases their deformities. Oxidation of fatty acids in the head of the sperm leads to a loss of fluidity of the cytoplasmic membrane and a decrease in enzyme activity, which leads to an occurrence in the head area. Increased MDA resulting from increased ROS due to oxidative stress resulting from cyclophosphamide leads to mitochondrial oxidation and loss of ATP, which causes weakness and damage to the axial filament, and this is the reason behind the deformation of the sperm tail and decreased motility [29, 30]. Hesperidin had a distinct role in reducing the rate of sperm abnormalities in groups of rats treated with both doses and the result was consistent with the study [31].

The reason for this is due to the action of chemical compounds and flavonoids, as they work to form non-covalent bonds within the lipid layers close to the plasma cell membrane, which greatly enhances its antioxidant activity and the renewal of endogenous antioxidants [32]. Flavonoids may interact with the polar head of phospholipids at the hydrophilic surface of the plasma membrane, thus protecting the membranes from oxidative damage [33]. The reason flavonoids protect sperm from deformities is due to the cell getting rid of hydroxyl radicals and nitric oxide, which cause DNA damage. Thus, flavonoids inhibit DNA damage [34]. Hesperidin regulates the GLP-1, PGCL-α, and PPAR-α genes that are necessary to maintain fertility, as they control sperm formation, raise sperm quality, and the metabolism process within the testicle [35, 36].

The results of the current study showed a significant decrease in the Testosterone level of laboratory rats treated with cyclophosphamide. This result was consistent with the study of [29] in which the decrease in the level of Testosterone of male laboratory rats after treatment with the drug cyclophosphamide confirmed its direct effect on Leydig cells. The toxic effect of the drug increases the free radicals that increase oxidative stress, which increases lipid peroxidation and interacts with the receptors on the membrane of Leydig cells, activating their cell death or causing histological and functional damage to the Leydig cells, thus negatively affecting the production of Testosterone in the plasma of laboratory

rats [37]. Testosterone is reduced by cyclophosphamide treatment via hypothalamic-pituitary-mediated action. It leads to the disruption of the signaling receptors responsible for the secretion of LH in the pituitary gland. This leads to a decrease in the LH hormone and a lack of stimulation of the Leydig cells to secrete Testosterone. That is, damage caused by negative feedback to the pituitary-pituitary axis [38]. Cyclophosphamide also caused a reduction in the number of LH receptors in the Leydig cells, which results in a decrease in the LH hormone stimulating the Leydig cells to synthesize Testosterone [39]. As for the protective role of hesperidin, the current study showed a significant increase in testosterone concentration in the treated groups compared to the group injected with cyclophosphamide. This result was consistent with the study of [39] where hesperidin stimulated the testicle, epididymis and the hypothalamus-pituitary stimulates the secretion of Testosterone through the regulation of steroidogenic genes, including StAR, CYP11A1, HSD17B, CYP 17A1, and CYP171A1, and the antioxidant capacity and enhancement of testicular functions. Flavonoids also work to prevent the inhibition of steroid synthesis and increase the secretion of gonadotropins [40].

The results of the histological study of the group treated with cyclophosphamide showed the occurrence of vacuolar degeneration in most of the seminal tubules and the absence of spermatogenesis in the lumen of some seminal tubules compared with the control group. It is also observed that vacuolar degeneration occurs in the Sertoli cells, the interspaces between the seminal tubules enlarge, the appearance of gaps between the Leydig cell clusters, and the sloughing of germ cells in the lumen of the seminal tubule. These results were similar to the study [13]. The reason behind these changes is the increase in ROS and free radicals, which cause an increase in oxidative stress and damage to the DNA of germ cells in the seminiferous tubule, which induces apoptosis and cellular degeneration, especially spermatocytes, which are highly susceptible to cytotoxins due to their rapid proliferation, while most Sertoli and Leydig cells remains alive but suffers functional damage from chemotherapy [41]. In addition, cyclophosphamide caused severe damage to Sertoli cells. The appearance of vacuolar degeneration is shown in the current histological study. The drug also oxidized cell membranes and damaged their DNA. It is known that Sertoli cells play a role in continuing the process of sperm formation by embracing, protecting and nourishing germ cells. Most germ cell divisions also take place between their protrusions, especially the primary spermatocyte and spermatids, which leads to a decrease in the number of germ cells [42]. The results of the current study showed a clear improvement in the components of testicular tissue, regularity in the size of the seminal tubules, the absence of large interspaces between the seminal tubules, the presence of Leydig cells in their natural shape between the seminal tubules, and the presence of the process of spermatogenesis compared to the treated group. Hesperidin played a role in disrupting the formation of free radicals, activating the enzymes SOD and GSH that combat oxidative activity caused by free radicals, and reducing drug toxicity by reducing the level of MDA and reducing fat oxidation. This is consistent with the study of [26]. Hesperidin reduces oxidative stress in Sertoli cells. Thus, protecting the germ cells in the seminiferous tubule and completing the spermatogenesis. Hesperidin also plays a prominent role in raising the level of Testosterone, thus increasing the number of sperm the seminal tubule [35].

TO THE FIRST PAGE NEXT PAGE PREVIOUS PAGE BACK TO CONTENTS

CONCLUSION

The results of the study confirmed the protective, improving and antioxidant role of hesperidin against the toxic effects of cyclophosphamide on testicular tissue and germ cells in rats treated with cyclophosphamide through increasing sperm numbers, reducing sperm deformities, activating the spermatogenesis and improving testosterone levels.

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Study of the Effect of Beta Vulgaris Aqueous Extract and Hydroxyurea on Testosterone and Erythropoietin Hormones in Female Laboratory Rats Induced with Phenylhydrazine

Conflict of interest: nothing to declare.
Authors' contribution: Ayat Mohammed Muhaibis Awas – conceptualization, data curation, investigation, methodology, project administration, resources, software, writing – original draft and writing – review & editing; Zainab Hudhi Farhoud – conceptualization, methodology, resources, supervision, validation, writing – original draft and writing – review & editing.
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Abstract

Introduction. Testosterone is a steroid hormone that affects tissue growth and reproductive functions found in both males and females. Erythropoietin (EPO) is a primary hormone responsible for stimulating the bone marrow to produce red blood cells, and is secreted mainly in response to low blood oxygen.
Purpose. The study was conducted to investigate the effect of beetroot extract and hydroxyurea on Female rats and then induce hemolytic anemia using Phenylhydrazine.
Materials and methods. The groups were divided into seven groups, each group containing 5 Females. The first control group was injected with distilled water, the second group was injected with Phenylhydrazine 0.1 mg/kg, the third group was dosed with beetroot extract 100 mg/kg. The fourth group was dosed with a mixture of beetroot extract and hydroxyurea, the fifth group was injected with Phenylhydrazine and dosed with beetroot extract, the sixth group was injected with Phenylhydrazine and a dose of hydroxyurea, the seventh group was dosed with a mixture of hydroxyurea and injected with Phenylhydrazine and beetroot extract.
Results. Phenylhydrazine inhibited erythropoietin production but increased testosterone levels, which may be attributed to its effect on hemolytic anemia and the body's compensatory response. Hydroxyurea reduced erythropoietin levels but did not show a significant effect on testosterone. In contrast, beetroot extract enhanced erythropoietin production without a significant effect on testosterone.
Conclusion. The chemical effects of both PHZ and hydroxyurea may be related to changes in bone marrow function and red blood cell production, while beetroot extract may help improve blood oxygen levels by enhancing EPO production. However, these components have no effect on sex hormone Testosterone.
Keywords: beta vulgaris, hydroxyurea, erythropoietin hormone, testosterone hormones, phenylhydrazine

INTRODUCTION

Hormones are biological compounds that play a vital role in regulating various body functions, including blood cell production and endocrine activity. Among these hormones, erythropoietin (EPO) is a primary hormone responsible for stimulating the bone marrow to produce red blood cells, and is secreted mainly in response to low blood oxygen [1]. On the other hand, testosterone is a steroid hormone that affects tissue growth and reproductive functions, and is present in both males and females but at varying levels. The levels of these hormones are affected by several factors, including chemotherapy, medications, and herbal supplements. Among the compounds that affect the production of these hormones is Phenylhydrazine (PHZ), a chemical used to induce hemolytic anemia in research models, as it destroys red blood cells, which may stimulate or inhibit the production of erythropoietin [2]. Hydroxyurea is a drug used to treat certain blood disorders such as sickle cell anemia, and affects the production of red blood cells [3]. In contrast, beetroot extract (Beta vulgaris extract) is known to boost nitric oxide production, which improves blood flow and may influence hormone levels [4].

This study aims to analyze the effect of Phenylhydrazine, hydroxyurea, and beetroot extract on erythropoietin and testosterone levels in females, helping to understand the biochemical mechanisms behind these changes.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted on 35 Female laboratory rats, distributed into seven groups, each group containing five rats as follows:

The first group, which is the control group, the rats in this group were given a standard diet with drinking water and were given distilled water for 30 days.

The second group: The rats in this group were injected with PHZ subcutaneously with an intraperitoneal needle at a dose of 0.1 mg/kg over 48 hours, in addition to the standard diet accompanied by drinking water.

The third group: This group was treated with the aqueous extract of beetroot 100 mg/kg orally in addition to the standard diet with drinking water throughout the experiment period.

The fourth group: This group was given hydroxyurea at a rate of one ml/kg orally on a daily basis in addition to the standard diet with drinking water throughout the experiment period.

Group Five: This group was given PHZ at a concentration of 0.1 mg/kg of body weight/48 hours in addition to being given orally with aqueous extract of beetroot at a concentration of 100 mg/kg in addition to the standard feed with drinking water throughout the experiment period.

Group Six: This group was given PHZ at a concentration of 0.1 mg/kg of body weight/48 hours in addition to a dose of hydroxyurea at a rate of one ml/kg orally on a daily basis, in addition to the standard feed with drinking water throughout the experiment period.

Group Seven: This group was given PHZ at a concentration of 0.1 mg/kg 48, in addition to aqueous extract of beetroot at a concentration of 100 mg/kg, in addition to the drug at a rate of one ml/kg orally on a daily basis in addition to the standard feed with drinking water throughout the experiment period.

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

Collection of Beetroot

Beetroot was obtained from the local market in Nasiriyah city in the winter of 2023, where the plant was taken and washed with sterile distilled water, then dried on filter paper at laboratory temperature, then crushed and ground using an electric grinder, and stored in sterile, tightly sealed containers in the refrigerator (4 °C) until used for extraction.

Preparation of Beetroot Aqueous Extract

The extract was prepared by soaking, where 10 grams of the beetroot powder subject to the study were taken and placed in 100 ml of sterile distilled water for two hours, and at the same time soaking for 24 hours and the solids were removed by filtration. The extract was passed over filter paper, then centrifuged using a Hettich universal device at a speed of 3000 rpm for ten minutes, then the filtered liquid was taken and placed in sterile bottles and stored in the refrigerator until use.

Determination of the Effective Dose

The most effective dose of the German extract of the beetroot plant Beta vulgaris was determined. 20 animals were used and divided into four groups, with 5 animals in each group, and they were distributed as follows:

The first group: (the control group was injected with distilled water).

The second group only: (was injected with 50 mg/kg of body weight of the aqueous extract of the plant).

The third group: (was injected with 100 mg/kg of body weight of the aqueous extract of the plant).

Group 4: (Injected with 200 mg/kg body weight of beetroot aqueous extract and given the dose orally via tube feeding for each animal. After forty-eight hours, blood samples were taken using a capillary tube from all groups through the eye cavity, then the serum was separated using a centrifuge at 3000 rpm for five minutes [5].

This is to measure the hemoglobin concentration. In light of this, the most effective dose of the aqueous extract was chosen: 100 mg/kg body weight, as the effective dose showed a significant effect in reducing the hemoglobin percentage. Then the dose was repeated at the same concentrations above for 72 hours to ensure the effective dose, and the effective dose was at the same concentration of 100 mg/kg body weight.

■ RESULTS

A decrease in the level of Erythropoietin (EPO) in female laboratory rats in the group infected with PHZ-induced anemia compared to the control group. A significant decrease in the level of EPO ($P \leq 0.05$) in all groups treated with the medical drug Hydroxyurea when compared with the control group as shown in Fig. 1.

Testosterone showed no significant differences in female laboratory rats ($P \leq 0.05$) in all treated groups (Phylhydrazine) when compared with the control group. It showed no significant ($P \leq 0.05$) in all groups treated with the aqueous extract of the beetroot plant (Beta vulgaris extract) when compared with the control group. It showed no significant differences ($P \leq 0.05$) in all groups treated with the medical drug Hydroxyurea when compared with the control group as shown in Fig. 2.

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

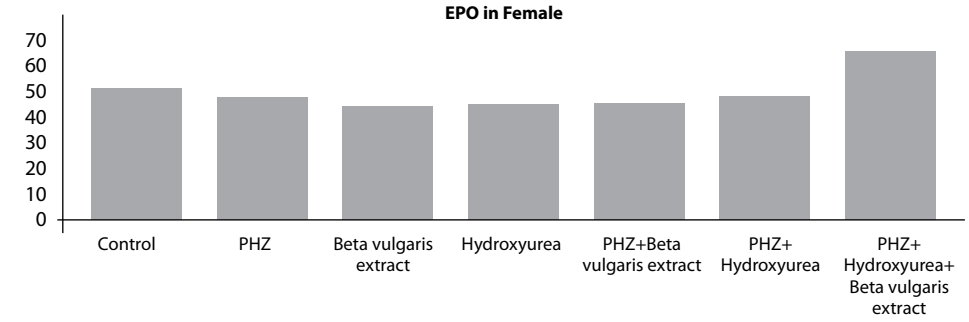

Fig. 1. The level of EPO hormone in groups treated with PHZ, Beta vulgaris extract and hydroxyurea

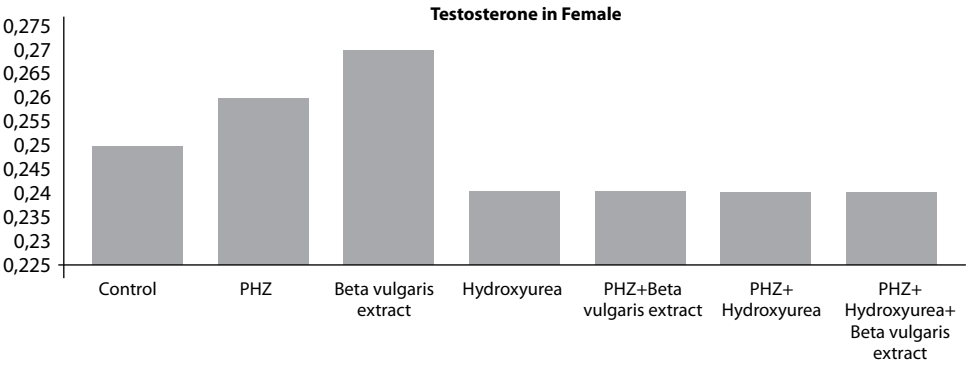

Fig. 2. The level of Testosterone hormone in groups treated with PHZ + Beta vulgaris extract and hydroxyurea

■ DISCUSSION

The results of the current study differed from the study [6] which indicated a decrease in the level of Testosterone in the group injected with PHZ as it is associated with increased production of free radicals, which in turn causes disturbances in the functions of Leydig cells and Sertoli cells. As Leydig cells cause an increase in oxidative stress, which leads to a decrease in the synthesis and secretion of the hormone Testosterone, and therefore the decrease in this hormone is an effective factor in causing disorders in sperm formation and a decrease in the number in the epididymis [7].

The results of the current study differed from the study [8] which indicated an increase in testosterone in the groups treated with the aqueous extract of the beetroot plant, because this extract contains strong antioxidants such as betalain, which contribute to reducing oxidative stress in the body. This may improve the overall hormonal balance, which enhances the body›s ability to secrete testosterone more effectively.

The results of the current study differed from the study [9] which indicated an increase in testosterone levels when treated with hydroxyurea as a result of the effect of this drug on regulating the endocrine glands as well as its effect on the production of hormones in the pituitary gland and reproductive glands. Hydroxyurea also stimulates the secretion of hormones that regulate testosterone such as luteinizing hormone (LH) which regulates testosterone production in the testicles.

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

The results agreed with the study [10] which showed that long-term use of PHZ causes chronic oxidative stress or kidney damage, which leads to a decrease in the production of the hormone EPO, thus not compensating for the deficiency in red blood cells. The results of the current study also showed a decrease in the level of the hormone EPO in female laboratory rats in the group treated with the aqueous extract of the beetroot plant Beta vulgaris extract at a probability level ($P \leq 0.05$) compared to the control group. The results of the current study agreed with the study [11] which showed that the aqueous extract of the beetroot plant Beta vulgaris extract is rich in nitrates which are converted into nitric oxides (NO) in the body, which leads to the expansion of blood vessels and blood flow, which leads to the delivery of oxygen to the tissues and thus reduces the stimulation of the kidneys to secrete erythropoietin, which leads to a decrease in the level of the erythropoietin hormone. The results agreed with the study [12] which indicated a decrease in the level of EPO hormone in groups treated with the medical drug hydroxyurea because it works to directly inhibit the proliferation of red blood cells in the bone marrow, and this effect leads to a decrease in the stimulation of the production of erythropoietin hormone. This current study also agreed with the study [13] which showed that hydroxyurea can contribute to reducing the need to stimulate the production of red blood cells, and thus may cause a decrease in the level of erythropoietin hormone.

■ CONCLUSION

The chemical effects of both PHZ and hydroxyurea may be related to changes in bone marrow function and red blood cell production, while beetroot extract may help improve blood oxygen levels by enhancing EPO production. However, these components have no effect on sex hormone Testosterone. The development of new therapeutic strategies for anemia and hormonal disorders through the use of natural supplements or the control of drugs that affect blood production.

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The Association Between Serum Level of Protein C and Protein S and Antithrombin III Level in Women with Recurrent Miscarriage

Conflict of interest: nothing to declare.
Authors' contribution: Rania Judi Taref – conceptualization, data curation, investigation, methodology, project administration, resources, software, writing – original draft and writing – review & editing; Maysoon Sharief – conceptualization, supervision, validation, visualization, writing – original draft and writing – review & editing.
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Abstract

Introduction. Several studies have reported that thrombophilia is responsible for recurrent pregnancy loss (RPL).
Purpose. The aim of this study was to evaluate the prevalence and role of inherited thrombophilia in early pregnancy loss, specifically in the first trimester.
Materials and methods. A total of 104 women (patients) with a history of 2 or more miscarriages during the 1st trimester of pregnancy and 110 women (controls) who had experienced 2 or more births without a miscarriage were included in this study. In both groups, we determined the biological activities of antithrombin III (ATIII) and protein C (PC) using the chromogenic method and the biological activity of protein S (PS) were examined using a clotting method.
Results. In the patient group, deficiencies of ATIII, PC, and PS were detected in 3 (2.88%), 4 (3.85%), and 6 (5.77%) cases, respectively. In the control group, ATIII (0%) deficiencies were not detected, and deficiencies for PC (0.9%) and PS (0.9%) were each detected in 1 patient. APCR was detected in 9 patients (8.65%) and 4 control subjects (3.63%).
Conclusion. Based on our results, we can conclude that thrombophilia is a causal factor for miscarriages in the 1st trimester of pregnancy, although there are the conflicting data in the literature.
Keywords: antithrombin III, miscarriage, protein C, protein S, thrombophilia

■ INTRODUCTION

Based on a global assessment, it is estimated that there are around 23 million cases of spontaneous pregnancy loss on an annual basis [1]. One to two percent of pregnancies in the mid trimester are ended before the 24-week stage [2]. The significant importance for women is of concern and therefore, it is essential to conduct a thorough examination for every instance of pregnancy loss in order to ascertain the precise etiological reasons and associated risk factors [3].

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

Mutations occurring in the genes associated with (also known as the prothrombin gene), and genetic conditions that contribute to hereditary thrombophilia and spontaneous miscarriages [4]. Furthermore, deficiencies in antithrombin III (ATIII), protein C (PC), or protein S (PS) are significant factors contributing to thrombophilia, a condition characterized by an increased susceptibility to venous thromboembolisms during pregnancy [5].

Thrombophilia is a group of conditions that contribute to an increased susceptibility to blood hypercoagulability and subsequently lead to the occurrence of thrombosis especially the uteroplacental circulation. Thrombophilia may arise for several reasons, including the presence of the prothrombin mutation deficiencies [6]. The hereditary factor V Leiden is a prevalent form of thrombophilia that is often linked to recurrent pregnancy loss (RPL) [7].

Antithrombin (AT) is a small protein molecule that serves as an inhibitor for a range of enzymes within the coagulation cascade. The presence of an antithrombin III deficiency is a potential risk factor for recurrent pregnancy loss (RPL). Protein C and S deficiency are uncommon, hereditary conditions that have a higher risk of developing thrombophilia and eventually placental insufficiency and miscarriage. However, this hypercoagulation may result in problems for both the mother and the foetus, including pre-eclampsia, placental abruption, foetal growth restrictions, and recurrent pregnancy loss (RPL) [8].

So, relationship between blood disease and repeated abortion remains questionable. Therefore, the aim of this study is to evaluate the prevalence and the role of inherited thrombophilia in recurrent pregnancy loss.

■ MATERIALS AND METHODS

This is case control study which was done in Maternity and Child Hospital and include 150 women with history of 2 or more miscarriages in the 1st trimester as patient group. Second group include females had one or more live births. Both groups were matched by age. Females with history of spontaneous abortions in the first trimester of pregnancy should had the following investigations like chromosomal study, TORCH infections, immunological test, and endocrine assessment like TSH, T3, random blood sugars and hyperprolactinemia. The exclusion criterion for both groups was current pregnancy. In both groups, we determined the biological activities of antithrombin III (ATIII) and protein C (PC) and Factor V Leiden (FVL), using the chromogenic method and the biological activity of protein S (PS) will be examined using a clotting method.

Blood samples were collected with 3.2% sodium citrate in a 9:1 blood to citrate ratio. They were centrifuged at 2000 rpm for 15 min to separate the plasma, which was quick-frozen and maintained at –35 °C until testing. The study was approved by the Ethical Committee of the Iraqi Board for Medical Specialization. Written informed consent before inclusion in the study were provided for all patients as well as the control groups.

Full screening tests for hemostasis: The Prothrombin Time, Activated Partial Thromboplastin Time, and Thrombin Time (ATIII) were performed for all subjects.

Statistical analysis

The odds ratio (OR) and 95% confidence interval were estimated separately for each inhibitor, and APCR was analyzed with the Chi-square test in Instate 3.0 software (GraphPad Software, San Diego, CA, USA). Data were compared by using the t-test and the difference was significant if $P<0.05$.

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

RESULTS

In this study 60 women (the patient group) had 2 abortions before 12 weeks pregnancy and in 70 females (the control) with high parity. Mean age for patients was 30.3±23 years with a range of 20–42, in comparison to the mean age of the control women which was 31.5±21 years with a range of 21–40. Among patients' group, 34 (56%) females had 2 abortions, 15 (31.60%) had 3 abortions and 11 (10.53%) with more than 3 abortions (Table 1).

Table 1
Rates for abortions

No.	Group (n=60)	%
2	34	56
3	15	31.6
>3	11	10.53
1st trimester abortion	39	
2nd trimester abortion	21	

The estimation for antithrombin, protein c, protein S, deficiencies showed in Table 2. Among patients, the estimation of antithrombin, protein C, protein S decrease and showed as: 2.88% (3/60), 3.85% (4/60), 5.77% (6/60), and 2.88% (3/60) respectively. While among the control females, antithrombin decreased to 0% (0/104) in addition to 0.9% in both protein C and protein S (1/60). Values reported for antithrombin, protein C, and protein S decrease were different between patients and control women (Table 2).

Table 2
The decreased values for antithrombin III, protein C and protein S in the women with disease and in the non-disease

Variables	Disease females (n=60)	Non diseases (n=70)	Odds ratio (95% CI)	P value
ATIII	3	0	6.67 (0.78–55)	0.05
Protein C	4	1	4.36 (0.48–39.7)	0.04
Protein S	9	1	6.67 (0.78–56)	0.04
Factor V Leiden (FVL)	6	2	2.51 (0.27–8.41)	0.05

There were 71.1% (39) of patients had abortions occurred in the first trimester (<13 weeks of gestation), and 13 patients (28.9%) had abortions occurred in the second trimester (≥13weeks of gestation). There was significant association of low protein C with the abortion in the second trimester (P<0.01 respectively) (Table 3).

Table 3
The association of gestational age with protein C & protein S

Variable	Gestational age <13	Gestational age >13	P value
Protein C	0	3	0.01
Protein S	1	5	0.01
Protein S&C	4	3	0.1

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

■ DISCUSSION

For both patients and obstetricians, recurrent pregnancy loss is heartbreaking and disappointing because a causative etiology cannot be found in around half of the cases.

Maternal thrombophilia has recently been identified as one of the causes of adverse pregnancy outcomes, including recurrent pregnancy loss, still-birth, severe pre-eclampsia, placental abruption, and intrauterine growth restriction [9]. However, deficiencies of the natural anticoagulant like protein C, S and antithrombin occur much less than 1% to 2% in RPL patients. The incidence of hereditary hemophilia in the community is low in comparison with women with RPL [10].

In this study, most of the pregnancy loss cases who had Protein C and protein S deficiency occurred during 1st trimmest. In contrast, Alshammary et al. [11] has found Protein C and protein S deficiency had a significant association with 2nd-trimester pregnancy loss.

The present study as well as Jyotsna et al. [12] have found a significant association in the low mean value of protein C in the patient group compared with the control group. They believed that protein C deficiency may increase the risk of pregnancy loss but the incidence of miscarriage and stillbirth in women with severe protein C deficiency is unknown. In contrast, other studies have reported that protein C had no significant difference between patients and controls [13, 14].

A significant association of RPL with protein S deficiency was found in the present study, which is in agreement with others [15, 16]. Moreover, PS deficiency was also found associated with late-term fetal loss [14]. Moreover, In the European Prospective Cohort on thrombophilia study, which involved 1384 women, authors explained that only the levels of free protein S fell significantly during the 1st and 2nd trimesters of pregnancy, but there is no further decrease during the third trimester. Protein S deficiency is more commonly identified than protein C deficiency, constituting an overall 15-fold increased risk of recurrent pregnancy loss. In addition, they have observed that there are 2 S proteins; type I protein S deficiency is a reduction in the level of free and total protein S. Type III deficiency is a reduction in the level of free protein S only. Type II deficiency is a reduction in the cofactor activity of protein S, with normal antigenic levels. Age affects total protein S but not free protein S levels [17, 18].

A meta-analysis studies have established that protein C deficiency has been associated with an increased risk of second-trimester miscarriage and stillbirth [18, 19].

The prevalence of protein C, and protein S deficiencies are higher in patients with thromboembolic events compared with those in the healthy population because deficiency of protein C and protein S results in the loss of these natural anticoagulant properties, resulting in unchecked thrombin generation and thromboembolism [20].

Since, the present study found a considerable number of protein C and protein S deficiencies among RPL pregnant women, therefore, screening and subsequent therapy for thrombophilia is required among pregnant women coming for check-ups in hospitals. There are contradictory hypotheses concerning the role of thrombophilia as a cause of abortions. According to many authors, abortions do not occur in all cases of thrombophilia [21]. Other reports [22, 23] have stated that the combination of more than one inherited thrombophilia gene defect has been recognized as a cause of early and late RPL. Despite that, the incidence of thrombophilia is not clear [24]. Moreover, Sibai et al. [25] found that

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

approximately 0.2–1% of patients with a combined deficiency of PC and PS have normal pregnancy outcomes.

It has been recorder in a study involved 10,000 blood donors, that the prevalence of type-1 antithrombin deficiency was 0.02% [20], whereas the prevalence of heterozygote PC deficiency in the general population is 0.3% (approximately 10 times more than the prevalence of AT) [14] which indicated that risk of pregnancy complication is more with protein C than with antithrombin III.

ATIII deficiency has long been associated with a significant thrombotic tendency throughout gestation. But it was demonstrated only recently that hereditary ATIII deficiency is associated with miscarriages and stillbirth. The risks are greatest for women with ATIII deficiency who have ATIII, PC, PS, and PC resistance [26]. Although we found that the overall percentages of cases with ATIII, PC, and PS deficiencies were higher in the patient group than in the control group, we are not certain whether the recurrent miscarriages in these patients were caused by a deficiency of ATIII, PC, or PS because approximately 70% of 1st-trimester spontaneous abortions (12 weeks) result from chromosomal disorders [27]. Additionally, a significant number of early pregnancy spontaneous abortions result from the presence of antiphospholipid antibodies, anatomical abnormalities, specific infections, and endocrine disorders [11, 12, 28]. There are contradictory hypotheses concerning the role of thrombophilia as a cause of abortions. According to many authors of RLP, abortions do not occur in all cases of thrombophilia [4].

There is a consensus regarding the association between thrombophilia and mid-trimester losses, but the link with 1st-trimester miscarriages remains controversial. When compared to patients without thromboembolic events, individuals with these conditions had higher rates of antithrombin, protein C, and protein S deficits. According to literature research, it has been shown that patients with thromboembolic events have, whereas 0.5–1% of these individuals have antithrombin (AT) deficiency. On the other hand, both protein C (PC) and protein S (PS) deficiencies have a comparable incidence of 3% among the same group of patients [4].

■ CONCLUSION

Even there is no clear causal relationship could be drawn, protein C and S deficiency could be one of the causative factors for recurrent pregnancy loss. Thus, defect in clotting factors is needed in the diagnosis of 1st and 2nd trimesters and should be done in every woman with unexplained pregnancy loss should be tested for defect in clotting factors.

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TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

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Effect of IL-17 and IL-23 Levels in Women with Polycystic Ovary Syndrome

Conflict of interest: nothing to declare.
Authors' contribution: Rawaa Bannay Zubairi – conceptualization, data curation, investigation, methodology, project administration, resources, visualization, writing – original draft and writing – review & editing; Shireen Ali Hasan – conceptualization, data curation, investigation, methodology, project administration, resources, software, writing – original draft and writing – review & editing; Hayder Fadhil Okab – conceptualization, investigation, methodology, project administration, resources, software, visualization, writing – original draft and writing – review & editing.
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Abstract

Introduction. Polycystic ovary syndrome (PCOS) is a prevalent reproductive endocrine condition in women. Diagnosis is difficult and often delayed, and there are few therapy choices.
Purpose. This research was done to highlight the effects of the cytokines IL-17 and IL-23 and the body mass index (BMI) in women with PCOS.
Materials and methods. The present study examined 64 women with PCOS and 64 women in a control group at the Infertility Unit of Al-Nasiriyah Teaching Hospital. The women's age range was 16–43 years. Enzyme-linked immunosorbent assay (ELISA) was used to evaluate the cytokines.
Results. The results showed a significant increase in IL-17, IL-23, and average BMI in women with PCOS in comparison to the control group. The groups showed a non-significant difference according to age group ($p=0.429$), but there were highly significant differences according to BMI categories ($p<0.001$). The PCOS group showed significantly higher rates of hirsutism, acanthosis nigricans, family history of PCOS, and married status compared with the control group. Employment status did not show significant differences ($p=0.154$).
Conclusion. Increased levels of IL-17 or IL-13 in association with increased BMI induce low-grade inflammation in the reproductive system that had effects on the ovulation process.
Keywords: PCOS, IL-17, IL-23, hirsutism, BMI

■ INTRODUCTION

About 5–15% of women throughout the world suffer from polycystic ovary syndrome (PCOS), making it the most common endocrinologic disorder in women and a leading cause of infertility [1]. The main symptoms of PCOS include excess body fat, insulin resistance or hyperinsulinemia [2], abnormal menstrual bleeding, and oligo-anovulation.

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

The main hormonal abnormalities are increased levels of oestrogen and androgens and decreased levels of progesterone [3].

In addition, PCOS is linked to a number of health issues. In comparison to the general population, women with PCOS have three to seven times as much risk of developing gestational diabetes, 10 times the risk of developing asthma, and three to five times the risk of experiencing recurrent miscarriage [4, 5]. The primary symptoms may change with age, and treatment must be individualized to meet the needs of women, which results in diagnostic and management hurdles in treating this mysterious disorder despite its prevalence [6].

Autoimmune illnesses may also be linked to PCOS. A correlation analysis has revealed that autoimmune disorders disproportionately affect women (78% of the affected population), which may be linked to oestrogen levels. One reason is that autoimmune disease tends to start earlier in women compared to males. Furthermore, ovarian function is controlled by communication between the ovaries, immune cells, and their substances, such as steroids, peptide hormones, prostaglandins, growth factors, and cytokines [3, 7]. The correlation between the female reproductive system and the immune system is widely acknowledged. Immunological factors, particularly pro-inflammatory cytokines, have a significant impact on many processes in the human ovarian follicles. These processes include folliculogenesis, corpus luteum formation, steroidogenesis, and the recruitment of leukocytes during ovulation [8].

Cytokines are small peptides that are secreted by many immune cells, including T helper cells, dendritic cells, macrophages, B cells, natural killer cells, and certain non-immune cells [9]. IL-17 is a group of inflammatory cytokines that are associated with inflammatory diseases and comprises six members: IL-17A, IL-17B, IL-17C, IL-17D, and IL-17E (also known as IL-25). The IL-17 family is primarily synthesized by Th17 cells. However, IL-17E is synthesized by Th2 cells and enhances the functionality of the Th2 pathway [10].

Obesity is associated with a connection between low-level systemic inflammation and the presence of macrophages in adipose tissue, as well as an increase in markers that promote inflammation. IL-17 is present in macrophages in adipose tissue and contributes to the onset of inflammation by promoting the release of IL-1β, TNF-α, and IL-6. IL-17A plays a role in inflammatory processes by influencing adipogenesis and glucose metabolism [11]. Research has indicated that pro-inflammatory IL-17 cytokines may be associated with infertility in women with PCOS [12]. IL-17 has a significant role in both adaptive and innate immunity. Furthermore, experimental research indicates that IL-17A may have a novel function in the neuroanatomical plasticity of the sympathetic autonomic system, which can occur with inflammation [13, 14].

There is ample evidence indicating the involvement of IL-17 in tissue inflammation. IL-17 causes the release of other cytokines that are pro-inflammatory and activate neutrophils. IL-17 is the primary cytokine produced by Th17 cells and boosts the activity of T cells while also triggering endothelial cells to secrete various pro-inflammatory substances, such as IL-1, IL-6, TNF-α, NOS-2, metalloproteinase, and chemokines. Consequently, it intensifies inflammation in endothelial cells [15, 16]. IL-17 is also responsible for the development of high blood pressure and impaired blood-vessel function caused by angiotensin II. Therefore, it can be regarded as a potential target for treating this condition [17].

IL-17 and IL-23 are cytokines that have essential functions in the immune system and the process of inflammation. They are mostly linked to the progression and upkeep of

persistent inflammatory disorders, such as autoimmune illnesses and certain forms of inflammatory bowel disease [18]. Recent research has indicated a possible association between IL-17, IL-23, and PCOS. Increased concentrations of IL-17 and IL-23 have been discovered in the bloodstream and ovarian tissues of women diagnosed with PCOS in comparison to women without PCOS [3].

These cytokines are believed to play a role in the persistent mild inflammation seen in PCOS. IL-17 facilitates the attraction and stimulation of immune cells, particularly neutrophils, and triggers the generation of other pro-inflammatory cytokines [19]. Conversely, IL-23 has a role in the specialization and longevity of a particular subset of T-helper cells known as Th17 cells, which are a primary producer of IL-17. Elevated concentrations of IL-17 and IL-23 in women with PCOS suggest their potential involvement in the initiation and advancement of inflammation in this disorder [18, 20]. Chronic inflammation can interfere with the normal functioning of the ovaries, resulting in irregular menstruation periods, the formation of ovarian cysts, and other symptoms commonly associated with PCOS [21].

The relationship between IL-17, IL-23, and PCOS is still an area of active research, and the precise processes through which these cytokines contribute to the development of PCOS are not yet completely comprehended. Further studies are needed to elucidate the precise roles of IL-17 and IL-23 in PCOS and to explore potential therapeutic targets for managing inflammation in this condition. Therefore, this research was conducted to highlight the effects of IL-17, L-23, and body mass index (BMI) in women with PCOS.

■ MATERIALS AND METHODS

Sample collection

A total of 128 blood samples were collected for this investigation from women with PCOS who were diagnosis by gynaecologists at the Infertility Unit of Nasiriyah Teaching Hospital according to previously published criteria [22]. About 3 ml were collected from each sample, placed in a gel tube, and left at room temperature for about 30 minutes. The gel tubes holding the samples were subjected to centrifugation at 4000 revolutions per minute for 5 minutes. The resulting serum sample was then separated into two identical portions and transferred into Eppendorf tubes.

Each portion contained approximately 400 µl of the sample and was stored in deep freeze at a temperature of –20 °C. The serum samples were utilized to assess the levels of cytokines IL-17 and IL-23 using the enzyme-linked immuno-sorbent assay (ELISA) technique. Analyses were done to examine associations with age, BMI, work type, hirsutism, family history, acanthosis nigricans, and marital status.

Statistical analysis

Data were analysed using the Statistical Package for the Social Sciences (SPSS) version 26. An independent-sample t-test was used to compare averages between patients and the control group, and a Pearson correlation analysis was used to determine the relationship between cytokines. A chi-squared test was used to check for independence, and a receiver operating characteristic (ROC) test was used to provide predictive values for the level of cytokines for discrimination between susceptible women and diseased women and according to body mass.

■ RESULTS

Demographic Characteristics of PCOS women and control Group

There was no significant difference between the two groups in terms of the average age and the age groups. There was a significant increase in BMI in the PCOS group in comparison to control group. In the PCOS group, 46.88% were overweight, 17.19% were obese, and 15.63% had obesity class 1, while the rates in the control group were 23.44%, 9.38%, and 0.0%, respectively. There was no effect of occupation on disease events.

Hirsutism occurred in 8.94% of women with PCOS, and 62.5% had acanthosis nigricans, while the rates in the control group were 20.31% and 10.94%, respectively. In addition, 92.19% of women with PCOS were married, and 64.06% had a family medical history of PCOS, as shown in Table 1.

Table 1
Demographic characteristics of PCOS women compared with control group

		PCOS No. 64		Control No. 64		p. value
		Mean±S.D.				
Age		29.07±7.74		28.90±7.36		0.898
BMI		29.65±6.11		23.12±4.35		<0.001
Categories		No.	%	No.	%	
Age	16-25 years	22	34.38	19	29.69	0.429
	26-35 years	25	39.06	31	48.44	
	36-43 years	17	26.56	14	21.88	
BMI	Under wight	2	3.13	21	32.81	<0.001
	Normal weight	11	17.19	22	34.38	
	Over weight	30	46.88	15	23.44	
	Obesity	11	17.19	6	9.38	
	Obesity 1	10	15.63	0	0.00	
Employed		24	37.50	32	50.00	0.154
Non-employed		40	62.50	32	50.00	
Have hirsutism		55	85.94	13	20.31	<0.001
Have not		9	14.06	51	79.69	
Married		59	92.19	45	70.31	0.002
Single		5	7.81	19	29.69	
Have FH		41	64.06	0	0.00	<0.001
Have not FH		23	35.94	64	100	
Have AN		40	62.50	7	10.94	<0.001
Have not AN		24	37.50	57	89.06	

Notes: FH – Family history; AN – Acanthosis nigricans.

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

Evaluation of IL-17 and IL-23 in PCOS women and control group

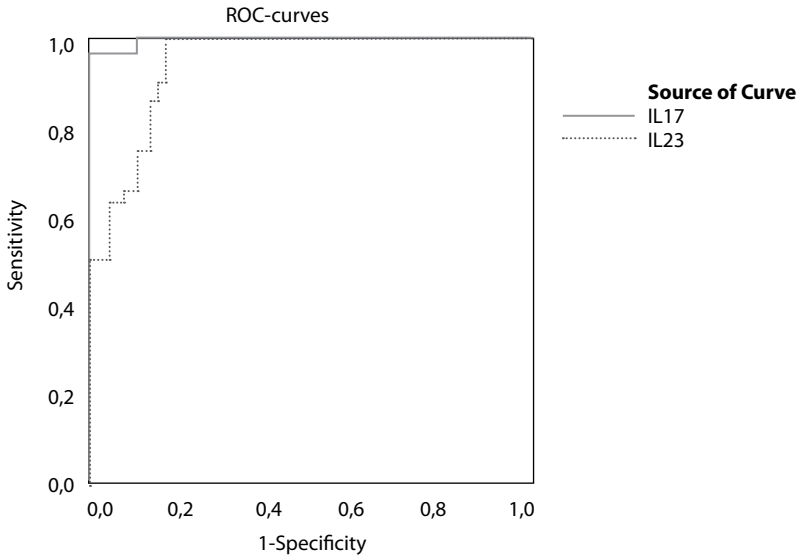
There was a significant increase in both IL-17 and IL-23 levels in women with PCOS compared with the control group ($p<0.05$), as shown in Table 2.

Table 2
Level of IL-17 and IL-23 in PCOS and control group

	PCOS	Control	p. value
	Mean±S.D.		
IL-17	44.81±14.8	15.02±4.60	<0.001
IL-23	38.62±7.64	21.88±6.48	<0.001

ROC Test

Both cytokines were analysed with an ROC test to determine the accuracy of the level of cytokine elevation in predicting PCOS compared to the same women from the control group. The test gave more than 99% correct positive results, as shown in Figure and Table 3.



The diagnostic precision of IL-17 and IL-23 was evaluated using the receiver operating characteristic (ROC) curve to compare PCOS patients with the control group

Table 3
Area under curve for IL-17 and IL-23

				CI 95%	
	AUC	S.E.	p. value	Lower	Upper
IL-17	0.997	0.003	<0.001	0.991	1.00
IL-23	0.943	0.019	<0.0010	0.906	0.981

TO THE FIRST PAGE NEXT PAGE PREVIOUS PAGE BACK TO CONTENTS

■ DISCUSSION

PCOS is a multifaceted endocrine illness that impacts various elements of a woman's health, such as reproductive, metabolic, and endocrine processes. Several factors, have been implicated in the pathophysiology, including immune system dysregulation, obesity (as indicated by BMI), and hirsutism (excessive hair growth). Recently, there has been increased focus on the significance of cytokines such as IL-17 and IL-23 in PCOS [23]. The current results show that IL-17 and IL-23 were significantly higher in women with PCOS than the control group. The rates of BMI, hirsutism, and acanthosis nigricans were also significantly increased in women with PCOS compared with the control group.

Studies have suggested that elevated levels of IL-17 may contribute to the development and progression of PCOS [24]. Also, a previous study showed that increased IL-17 levels are associated with insulin resistance, hyperandrogenism, and ovarian dysfunction in women with PCOS. IL-17 may also contribute to chronic low-grade inflammation, which is a characteristic feature of PCOS [25]. T cells play a role in promoting follicle maturation within the immune system [26]. IL-17 is expressed by cumulus cells, and the Bio Plex Protein Array System was used to verify the release and build-up of IL-17 protein from cumulus cells and granulosa cells [27].

IL-17 promotes the synthesis of IL-1β, which can initiate the maturation of cytoplasm and facilitate the acute inflammatory phase required for ovulation. IL-17 primarily carries out its immune regulatory role by stimulating the production of proinflammatory cytokines and chemokines, which attract neutrophils and macrophages to the site of inflammation [28]. This may explain its crucial function in the process of ovulation. Infertility has been associated with an increase in the expression of pro-inflammatory cytokines by granulosa cells [29].

Previous findings indicate that alterations in the levels of important cytokines that regulate folliculogenesis in the follicular fluid suggest the presence of ongoing mild inflammatory responses. These responses have a detrimental effect on folliculogenesis and the success of in vitro fertilization (IVF) treatment in women. During the window of implantation, the endometrium exhibits a proinflammatory condition. IL-17 stimulates the synthesis of IL-1β, which enhances the transmission of pro-inflammatory signals during the window of implantation [30]. IL-17 stimulates the synthesis of IL-6, and the highest levels occur during the implantation period [31]. IL-6 is synthesized by stromal and epithelial cells, and its production peaks during decidualization.

These findings indicate that IL-6 has a significant function in the decidualization process. IL-17 primarily carries out its immune regulatory role by stimulating the production of pro-inflammatory cytokines and chemokines, which attract neutrophils and macrophages to the site of inflammation [32]. These examples demonstrate its function in the inflammatory environment of the endometrium during the period of endometrial receptivity. The decidual transformation of stromal cells in women initiates prior to implantation and is facilitated by adjacent dendritic cells.

IL-23 is another pro-inflammatory cytokine that is involved in the activation and maintenance of Th17 cells. IL-23 has been found to be elevated in women with PCOS and may promote the production of IL-17 [33]. Studies suggest that the IL-23/IL-17 axis is implicated in the development of insulin resistance, ovarian dysfunction, and follicular abnormalities in PCOS. Increased IL-23 levels may also contribute to the chronic inflammation observed in PCOS [34, 35].

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

BMI, hirsutism, and acanthosis nigricans are also implicated in PCOS. Women with PCOS often have a higher BMI compared to women without PCOS [36], as shown in this study. Obesity and excess body fat indicated by higher BMI are commonly associated with PCOS. Higher BMI in PCOS is linked to insulin resistance, hyperandrogenism, and menstrual irregularities [37]. Hirsutism in the form of excessive hair growth in a male-like pattern is commonly observed in women with PCOS. The primary factor is commonly attributed to an increase in the production of androgens by the ovaries or a disturbance in the processing of androgens. Hirsutism occurs as a result of increased androgen levels in persons with PCOS [38, 39].

Acanthosis nigricans is a dermatological condition characterized by the appearance of black, thickened, and velvety patches of skin and can manifest as one of the related symptoms in women with PCOS, as demonstrated in this study. Insulin resistance is a medical disorder characterized by reduced sensitivity of the body's cells to the actions of insulin, resulting in increased amounts of insulin in the bloodstream. Insulin resistance is a frequent occurrence in women who have PCOS. High insulin levels can stimulate the growth of skin cells and increase the production of certain substances that cause acanthosis nigricans.

The dark, velvety patches associated with acanthosis nigricans typically appear in areas such as the neck, armpits, groin, and sometimes the hands and feet. These patches may be symmetrical and may gradually increase in size over time. While acanthosis nigricans can occur in individuals without PCOS, its presence in women with PCOS may serve as a clinical indicator of insulin resistance. Managing it involves addressing the underlying hormonal and metabolic imbalances. It is also crucial to make lifestyle changes such as maintaining an optimal weight, regularly participating in physical activity, and adhering to a well-balanced diet.

■ CONCLUSION

Increased levels of IL-17 or IL-13 in association with increased BMI induce low-grade inflammation in the reproductive system that had effects on the ovulation process.

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Mothers' Knowledge and Practice on Optimal Complementary Feeding

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Abstract

Introduction. Optimal complementary feeding during first two year of life is crucial for optimal development and growth of infant and young children during first two years of life.
Purpose. The goal of this study is to investigate mothers' knowledge and practices about the complementary feeding among infants and young children age from four months to two years of life.
Materials and methods. A cross-sectional study was designed to interview 210 mothers of infant and young children, aged from 4 month to two year who attended outpatient pediatrics clinic in Al-Mawani Teaching Hospital in Basrah, the relation between mothers' age and educational level, with their knowledge and practices about complementary feeding was evaluated.
Results. The results revealed only 28.57% of the participated mothers know the proper time of starting complementary food, and about 52% of the mothers start complementary food before six months of age. We didn't found statistical significant relation between mothers' age, mothers' education level and mothers' knowledge and practices, apart from a significant relation between level of mothers' education level and correct frequency of introduction new food per week (p-value 0.001).
Conclusion. There is a need to promote optimal feeding practices among mothers to insure optimal complementary feeding.
Keywords: complementary feeding, feeding practices, passive immunity, nutritional requirements, infants

■ INTRODUCTION

The process of giving of appropriate food at the proper time in addition to mothers' breast milk to face increased needing nutrients for the growing child is called complementary feeding. The exclusive breast feeding of the babies during the first six

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

months of life endows the baby a passive immunity that is essential to protects them from various bacterial and viral infections with the lack of a well-developed immune system at this critical period of time. In addition to that, the infants and young children who are provided with complementary food after the first half year will meet the rising nutritional requirements necessary for development and growth. This process called weaning, and mothers who do weaning should be knowledgeable about the timing and types of complementary feeding [1, 2].

In addition to that first two years of life considers as a critical period of time for development and growth of children. Most cases of malnutrition occur during this time, thus appropriate nourishment is critical. Malnutrition in the first two years of life can cause permanent impairment to physical and intellectual development. As a result, improving food habits during this time is critical to improve children's health and nutrition levels [3, 4].

Suboptimal complementary feeding practices are associated with a large burden of malnutrition and mortality in children [5].

Mother's information and work on with respect to reciprocal food play a significant part on their taking care of practices. The capacity of the mothers to do the suggested reciprocal taking care of practices is related with their insight and demeanor on ideal integral taking care of. Numerous variables could influence mothers' information and disposition towards complementary feeding [6–8].

The goal of our study is to determine the knowledge and practices of the mothers about complementary feeding and to identify the factors like mothers' education and mothers' age which could be affecting their knowledge and practice of complementary food.

■ MATERIALS AND METHODS

Study design

A cross-sectional descriptive study was carried out among mothers who visited the outpatient department of Al-Mawani Teaching Hospital in Basrah, over the period of four months, from January 2023 to April 2023.

Sample size

210 mothers of up to two years children were included in this study aged (17–44 years) who visited the pediatrics outpatient clinic in Al-Mawani Teaching Hospital in Basrah during the study period.

Exclusion criteria

Mothers of infants aged less 4 months or more than 24 months were excluded.

Inclusion criteria

Mothers of infants and young children between 4–24 months of age visiting the outpatient clinic of pediatrics were involved in this study.

Data collection

The study is based on interviews using questionnaire, data were included information about age of starting complementary food, type of food start with, causes of delay

complementary feeding, correct frequency of introducing new foods per week, and the way of feeding whether by the mother or baby-led which means babies are allowed to feed themselves.

Other variables addressed were the age of the mother, educational level and occupation of mother, family size, child age and sex and feeding pattern of child whether breast, bottle or mixed feeding.

Ethical approval

The study protocol received approval from the College Council and Ethical Committee at Basrah College of Medicine, University of Basrah, with reference number 030411-036-2022 on 3/4/2022.

Data analysis

The data collected was analysed using SPSS version 23. Chi-square test was used and a $P<0.05$ is considered significant.

■ RESULTS

210 mothers were involved in this study, majority of the mothers were belong to age group 20–40 years (88.57%) and majority of the mothers were housewives (92.85%), and about 74% of the mothers had primary and secondary school education. 15.7% of the mothers were illiterate and only 10% have college and above education. About 57% of children included in this study were male, and about 43% of them were female. Regarding the age of children 39.05% of them belong to age group 6–12 month, and 7.62% aged less than 6 months and 25.71% belonged to age group 12–18 and 27.62 belonged to age group 18–24 month. About tow third of children (66.67%) are bottle fed, were the remaining children were breast and mixed feeding with 23.81% and 9.52% respectively (Table 1).

Age of starting of complementary feeding was less than 6 months in 52.38% of children, and at age of 6 months of age in 28.57% of children and older than 6 months in about 19.05% of studied children. Way of giving complementary feeding was by mother in 91.43% of children and by baby-led in only 8.57% of studied children. Correct frequency of introduction new food per week was found in only 29.5% of children while about 70.5% of children had incorrect frequency of introduction new food per week. Frequency of complementary feeding was twice a day in about 41.9% of children and thrice a day in 58.1 of children. There was no significant correlation between mothers' age and age of initiation of complementary feeding, way of giving complementary feeding, and correct frequency of introduction new food per week, p-value is 0.635, 0.969, 0.789 respectively. There was a significant correlation between mothers' education level and correct frequency of introduction new food per week ($p=0.001$). There was no significant correlation between mothers' education level and the way of giving complementary feeding, age of initiation of complementary feeding p-value was 0.09 and 0.747 respectively (Table 2).

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

Table 1
General and sociodemographic characters of the mothers and their children

Character	No.	%
Mothers' Age (year)		
<20	14	6.67
20–40	186	88.57
>40	10	4.76
Mothers' Education Status		
Illiterate	33	15.71
Primary	84	40.00
Secondary	72	34.29
College and above	21	10
Mothers' Occupation		
Housewife	195	92.85
Government Employee	14	6.67
Private Employee	1	0.48
Student	0	0
Sex of the child		
Male	120	57.14
Female	90	42.86
Childs' Age(month)		
<6 m	16	7.62
6–12	82	39.05
12–18	54	25.71
18–24	58	27.62
Childs' feeding pattern		
Breast	50	23.81
Bottle	140	66.67
Mixed	20	9.52

Table 2
Mothers' Knowledge and their practice about complementary feeding

Variable	No.	%
Age of initiation of complementary feeding		
<6 m	110	52.38
At 6 m	60	28.57
>6 m	40	19.05
Way of giving complementary feeding		
By Mother	192	91.43
Baby – Led	18	8.57
Correct Frequency of introduction new food per week		
Yes	62	29.52
No	148	70.48
Frequency of complementary feeding		
Twice a day	88	41.90
Thrice a day	122	58.10

■ DISCUSSION

Complementary feeding plays a crucial for healthy growth and development of infants and young children during the first two years of life [9].

According to World Health Organization guidelines, complementary feeding should begin at six months of age, and breastfeeding should continue until two years of age [10, 11]. Delayed beginning of complementary foods may enhance the risk of

malnutrition, immune diseases, and development of diabetes mellitus in later life in high-risk population [12].

In this study we investigated mothers' knowledge and practices toward optimal complementary feeding of children from the age 4–24 months in Basrah.

The study shows that the most of participated mothers were young housewives and had low education level, these results are comparable with other studies was done in Baghdad [13], Erbil [14], and Al-Najaf [15].

In this study 28.57% of mothers know the correct age of introduction of complementary feeding, which is lower compering with other regions universally [16].

In this study (52.38%) of the participated mothers start complementary feeding before age of 6 months. Many mothers start complementary feeding before the recommended age of 6 months [17] these no add benefits from this practice [18] this may also increase risk of infants' alimentary infections and may increase the incidence of childhood obesity in the future [19].

The majority of the involving mothers (70.48%) didn't had an idea about the correct frequency of introduction new food per week, this may be due to low educational level of the participated mothers.

Regarding the way of giving complementary feeding, we found that the majority of the babies (91.42%) was fed by the mother and only (8.58%) by baby-led method.

In our study there was no significant relation between mothers' age and age of initiation of complementary feeding, way of giving complementary feeding, and correct frequency of introduction new food per week. Also, there was no significant relation between mothers' education level and the age of initiation of complementary feeding, way of giving complementary feeding, apart from a significant relation between level of mothers' education and correct frequency of introduction new food per week, similar results were obtained in study done in Kosova [20].

There were some limitations of our study is that cross-sectional design and small-sized sample which collected from single site of Basrah, in spite of that this study can be used as a foundation for future researches.

■ CONCLUSION

Optimal complementary feeding during first two year of play a crucial role in healthy development and growth of infant and young children. Many factors influence mothers' understanding and practices on complementary feeding, including their age, educational level, and family socioeconomic status. There is a need to promote optimal feeding knowledge and practices among mothers to insure ideal complementary feeding.

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Knowledge and Practice About Family Planning: A Study Among Women Attending Primary Health Care Centers

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Abstract

Introduction. Every woman can maintain her health and the health of her children by using a safe and efficient family planning strategy. The demand for contraception methods is growing as the number of fertile women rises, even though over half of all couples in the developing world use it to postpone, space out, or restrict future pregnancies.
Purpose. To determine the women's knowledge and practices regarding family planning, and to find out the association between the knowledge of women and their Socio-demographic characteristics.
Materials and methods. A descriptive, cross-sectional observational study was conducted with 300 women aged 15 and 49 years at primary health care centres in Basrah (6 centers) from 1st Jan to 31st March 2022.
Results. Most of the women (81.7%) knew the benefits of family planning, and they knew at least a single kind of contraceptive method (98.3%), the most common source of information is health personnel (46.7%). 75% of respondents using either type of contraceptive, mostly modern methods and oral contraceptive pills usage is about 64.67% of women. The most common source of family planning services is private pharmacies (68.9%). Around half of non-users of family planning due to health reasons and side effects (48.0%), There is a significant association between the usage of contraceptives and the parity ($P<0.05$).
Conclusions. The survey found that women had a good knowledge of family planning. Excellent use of the traditional and modern techniques. In addition to other factors, health concerns were the primary reason why people did not utilize contraceptives.
Keywords: family planning, primary health care centers, reproductive life, contraceptive, fertility

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

INTRODUCTION

Fertility is the real bearing of kids by fertile women throughout their reproductive years [1]. Women, their families, and the global community can all benefit greatly from family planning programs [2]. Family planning ensures that each family member grows and matures to their greatest potential by helping families to get the children number that they desire with the right time and spacing [3].

Family planning is the intentional utilisation of contraceptive methods by a couple to decrease or space out the number of their children [4].

It enables couples to have as many children as they want at the time and spacing that works best for them. Still, the reasons and situations that influence the use of contraceptives are diverse and vary throughout women's reproductive years [5].

A very important issue, which affects the fertility of any community, is the Contraceptive Prevalence Rate (CPR) defined as the proportion of married women, aged 15–49 years, using any method eighter modern or traditional methods of contraception at a given period of time [6].

Unplanned pregnancies and illegal abortions are the results of unmet family planning needs. In low-income nations, this is frequently the primary cause of mother and infant death and has serious health and social implications [7, 8].

A key approach to reduce these detrimental health effects is family planning [9–10]. In low-socioeconomic nations, such interventions can lower maternal mortality by 44%, 90% of abortions will be avoided, 20% of pregnancy-related morbidity and 32% of maternal deaths will controlled, worldwide [7, 11].

Adolescent pregnancies are decreased, pregnancy-related health hazards are avoided, and HIV/AIDS and other STDs are less likely to occur when family planning is used [12].

Having access to family planning techniques improves women's financial situation, encourages education, and progressively gives them more authority, which leads to better living standards [13, 14].

Globally, only 32% of married women from low-socioeconomic nations presently utilize modern contraception methods [13].

Lack of education and awareness about the risk of getting pregnant, beliefs that their husbands or other family members are against family planning, religious beliefs, fear of contraceptive side effects, or inability to access family planning services are the main reasons why people do not use any family planning methods [15,16]. Even though family planning techniques are well understood in Nepal [15], Nigeria [17], and Pakistan, even in rural regions [18], there is poor practice of these methods.

Several factors, including natural or artificial, male or female, temporary or permanent, traditional or modern oral or injectable, or IUCDs, can be used to categorize family planning methods [19].

According to earlier research, the rate of contraceptive use was low for both modern and traditional methods [19].

Therefore, this study was carried out and the study aimed to determine the women's level of knowledge and practices regarding family planning concepts and methods and to identify the relationship between women's knowledge, practices and Socio-demographic characteristics.

MATERIALS AND METHODS

This study was a descriptive cross-sectional study, conducted at six primary healthcare centers in Al-Basrah for two months from the 1st Jan to 31st March 2022. A convenient sample of 300 women aged between 15 and 49 years was selected. The purpose and the objectives of the study have been clarified for the participants who participated in this study by the researcher stressing the issues of privacy and verbal consent had been obtained from each subject before enrolling in the study. The data were collected by using a questionnaire which was designed for the study through an interview technique. Inclusion Criteria include women in their reproductive age, currently non-pregnant, and married women attending the primary health care centers in Basra city (6 centers). While exclusion criteria include women, who refused to participate in this study (only 2 women), females who attended these primary health care centers before the age of 15 years or after 49 years, and single women.

Statistical analysis of the data was carried out using the SPSS program (Statistical Packages for Social Sciences – version 23). The results were tabulated. The data were presented as numbers and percentages. The chi-square test (χ^2) was used to detect the relationship between variables. Statistical significance was considered whenever the P value was less than 0.05.

RESULTS

The study included 300 respondents. Those who were 30–39 years old represented 38.0%, and the mean age was 29.6±7.59 years. Most of them were married in the age group 16–25 years old (80.3%). Most women have 1–3 child (60.3%). Women with primary or intermediate levels of education were 37.3%, 80.3% of the participants were housewives. The most of the participants with middle socioeconomic status (500–1 000 000 D.IQ) (67.0%) (Table 1).

Table 1
The studied women's socio-demographic characteristics

	No.	%
Age (years)		
< 20	2	0.7
20–29	112	37.3
30–39	114	38
40–49	72	24
Age at marriage		
<15	49	16.3
16–25	241	80.3
26–35	9	3
>35	1	0.3
Parity		
No child	5	1.7
1–3	181	60.3
4–6	98	32.7
7–10	16	5.3

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

	No.	%
Level of education		
Illiterate	80	26.7
Primary	112	37.3
Secondary	108	36.0
Occupation		
Housewife	241	80.3
Employed	59	19.7
Income		
<500	14	4.7
500–1000000	201	67.0
>1000000	85	28.3

As shown in Table 2, most women know the benefit of family planning to avoid pregnancy (81.7%). Most women know at least a single kind of contraceptive method (98.3%). 46.7% of respondents' source of information is health personnel. Two-thirds of respondents using contraceptives (75%).

Table 2
Information about the usage of contraceptive methods and their sources

Variable	No.	%
Know the benefit of family planning		
No	55	18.3
Yes	245	81.7
Know at least one type of contraceptive method		
No	5	1.7
Yes	295	98.3
Source of information		
health personnel	140	46.7
Relatives	119	39.7
Friends	41	13.7
User of contraceptive		
User	225	75.0
Non user	75	25.0
Total	300	100.0

There is 55.67% of respondents currently use modern contraceptive methods, while 19.33% of respondents use traditional methods, and the residual (25%) were non-users as shown in Fig. 1.

There is 64.67% of women use modern methods of family planning the oral contraceptive pills and 65.5% of respondents use traditional methods of family planning as withdrawal methods as shown in Fig. 2.

The most mentioned source of contraception methods is the local pharmacies (68.9%) as shown in table 3.

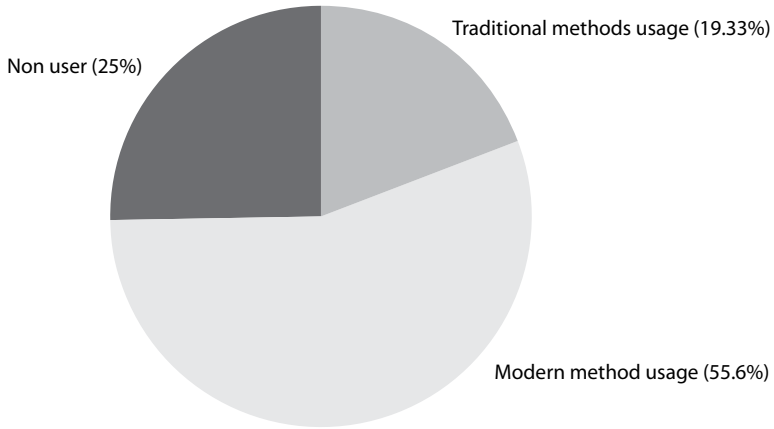

Fig. 1. The currently used family planning methods

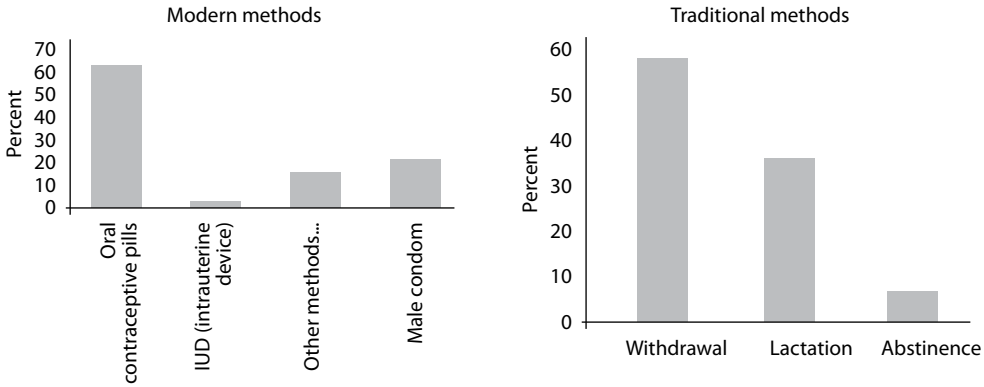

Fig. 2. Types of modern and traditional methods of family planning

Table 3
Source of contraception methods among studied women

Places to get contraception services	No.	%
Local pharmacies	115	68.9
Medical clinics	29	17.4
Local marketplaces	8	4.8
Public health centers	3	1.8
Governmental hospitals	12	7.2
Total	167	100.0

Around half of the non-users of family planning methods were due to health reasons and side effects (48%) as shown in Table 4.

Table 4
Reasons for refusing the contraception methods

Reasons for refusing the contraception methods	No.	%
Their health concerned and side effects	36	48.00
The desire for more children	19	25.33
Husband refusal	2	2.66
High cost	1	1.33
Religious convictions	2	2.66
More than one cause	14	18.66
Another reason	1	1.33
Total	75	100

Table 5 shows there is significant association was found between the user of contraceptives and women's parity (P=0.003).

Table 5
The relationship between contraceptive usage and socio-demographic characteristics of the studied population

Variables	User of contraceptive				
	User	%	Non-user	%	p-value
Age					
<20 years	2	100	0	0	0.854*
20–29 years	81	72.3	31	27.7	
30–39 years	87	76.3	27	23.7	
40–49 years	55	76.4	17	23.6	
Level of education					
Illiterate	63	78.8	17	21.3	0.593**
Primary	84	75	28	25	
Secondary	78	72.2	30	27.8	
Occupation					
Housewife	182	75.5	59	24.5	0.675**
Employed	43	72.9	16	27.1	
Income					
<500	8	57.1	6	42.9	0.074**
500–1 000 000	147	73.1	54	26.9	
>1 000 000	70	82.4	15	17.6	
No. of children					
No child	0	0.0	5	100.0	0.003*
1–3	135	74.6	46	25.4	
4–6	78	79.6	20	20.4	
7–10	12	75	4	25	

Notes: * Fisher's Exact Test; ** χ^2.

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

DISCUSSION

The study's population's fertility and demographic characteristics align with those of comparable earlier research conducted in Basrah city [20]. People in Basrah still favour early marriage, large families, and a role for mothers in the home, which may be caused by the unstable socioeconomic situation of our community. Other notable features include high rates of adolescent marriage, fertility rates, and low employment rates for mothers.

This study showed that most women know the benefit of family planning to avoid pregnancy (81.7%) similar to a previous study in Nigeria (85.8%) [21], which is higher than a previous study in Basrah (72.2%) [20] may be due to better health education by health personnel.

While 98.3% of women know at least one type of contraceptive method approximately similar to previous studies in Basrah (100%) [20] and Bagdad (100%) [22].

Information about family planning was mainly obtained from the health care provider (46.7%) and family members (39.7%) but still there is no big role for the media, similar to previous studies in Basrah [20] and Baghdad [22], this may be due to the low governmental role in health education of the general population about the essential role of family planning.

For many women, the source of contraception methods are mainly from local pharmacies (68.9%) which is similar to a previous study in Baghdad [22], and lower than Ebrahim and Muhammed study [20], and private clinics (17.4%) which higher than study [20], maybe due to, In Iraq high percentage of women buy the oral pills from private pharmacies as over the counter drug.

The usage of the modern method in the current study was (55.67%) which is similar to other studies such as the Egypt (53.9%), and Islamic Republic of Iran (56%), but higher than the previous study in Basrah [20], Azerbaijan (11.9%), Yemen (9.8%), and Sudan (7%) as mentioned by WHO references [23], may be due to the better health education last years.

However, in this study, women who are using traditional methods of contraceptives (19.33%) were more or less similar to a previous study in Basrah [20] (16.1%). Worldwide, the percentage of use of the traditional methods is lower than the use of modern methods [21, 24] may be due to in trust the use of traditional methods as type of contraception methods.

The non-users of contraception among the studied population mostly due to health reasons and side effects (48%) which is higher than the previous study in Basrah (44.4%) [20], similarly found in Mosul city north of Iraq [25]. This similarity might be the result of our participants, as any Iraqi woman may be more susceptible to the negative effects of modern contraceptives of any kind. This could be because of the conditions they have been living under for the past few decades, including low living standards brought on by multiple wars and sanctions that have affected all facets of life, especially for women of reproductive age, children, and the elderly.

A large proportion of women 25.33% don't practice family planning because they want more children which may be due to being forced by their husbands or their families to bring as many children as they want. Religious belief formed (2.7%) similar to a previous study in Basrah (4.1%) [20], almost approximately similar to a study reported in Nigeria (20%) [21].

Is significant association was found between the user of contraceptives and the number of children of women ($P>0.05$) may be due to young women's desire to become

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

pregnant soon after marriage because of societal pressures to prove their fertility and the improved status that motherhood gets, and when complete their family number then using contraceptive methods.

■ CONCLUSIONS

The study finds a good knowledge level of family planning. Good usage for both any method and mainly for modern types. The main cause for not using contraceptives was health reasons. There is significant association was found between the use of contraceptives and the parity of women.

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TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

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The Efficacy Satisfaction of Penile Prosthesis in Management of Erectile Dysfunction

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Abstract

Introduction. Erectile dysfunction (ED) significantly impacts the quality of life for affected individuals and their partners. Penile prosthesis implantation is widely regarded as the gold standard treatment for chronic ED, offering higher satisfaction rates compared to other therapies.
Purpose. This study aimed to compare the efficacy, safety, and patient satisfaction of two surgical techniques – subcoronal and infrapubic – for penile prosthesis implantation.
Materials and methods. A comprehensive evaluation of patient-reported outcomes, including sexual and psychosocial satisfaction, long-term erectile function, and complication rates, was conducted. Data were collected from a matched cohort, and advanced statistical analyses were employed to identify significant differences between the techniques.
Results. Both approaches are effective, but the subcoronal method showed lower complication rates and favorable cosmetic outcomes, while the infrapubic method offered advantages in reservoir placement and procedural ease. Advancements in prosthetic materials, such as hydrophilic coatings, and surgical innovations contributed to improved outcomes, including reduced infection rates and enhanced patient satisfaction.
Conclusion. While both techniques are viable, selecting the optimal approach should be based on individual patient needs, surgeon expertise, and desired outcomes. The findings contribute to bridging the knowledge gap and optimizing surgical strategies for penile prosthesis implantation, ultimately improving the quality of life for men with ED.
Keywords: infrapubic, sub coronal, erectile dysfunction, penile prosthesis, patient satisfaction

■ INTRODUCTION

A common condition that greatly affects both a man's and his partner's quality of life is erectile dysfunction Implantation [1]. The American Urological Association advises that all ED patients be made aware of the potential benefits of this surgical technique in light of these benefits [2]. Implanting a penile prosthesis has emerged as the benchmark of care for those with resistant ED or those who seek a permanent solution, despite the availability of other therapeutic choices [3]. When compared to other ED care strategies, studies

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

have consistently shown that patients and partners experience higher levels of sexual satisfaction after penile prosthesis various surgical procedures, including as penoscrotal, suprapubic, perineal, infrapubic, and subcoronal approaches, have been developed to improve the procedure's effectiveness and patient experience. Because the subcoronal technique has a low risk of problems, it has becoming more common, and pleasing cosmetic results. On the other hand, the Infrapubic technique has various drawbacks but has benefits in reservoir location. These methods are widely used, but there hasn't been a direct comparison of their results in terms of efficacy, safety, and patient satisfaction. This gap in knowledge highlights the need for additional study to determine the best surgical technique for implanting a penile prosthesis. The present study compares the sub coronal and infrapubic methods in individuals with ED in an effort to close this gap. Through an assessment of patient outcomes, such as safety, effectiveness, and psychological and sexual satisfaction, this research will evidence supporting choice the best surgical approach for penile prosthesis implantation [4, 5].

Few comparison studies comparing the results of various surgical implantation techniques [5], despite the recognized effectiveness of penile prosthesis in addressing erectile dysfunction and its related advantages in boosting sexual satisfaction for patients and partners. Although they have different benefits, the subcoronal and infrapubic techniques have not been directly evaluated with regard to patient-reported outcomes, safety, or efficacy [6]. Because of this dearth of information, physicians are unable to decide which surgical strategy would be best for any given erectile dysfunction patient [7]. In order to close this information gap, this study compares the subcoronal and infrapubic techniques to penile prosthesis implantation. The goal is to identify which route is better in terms of safety, effectiveness, and patient satisfaction.

Penile prosthesis research is important for treating erectile dysfunction. For a number of reasons, a thorough investigation contrasting the subcoronal and infrapubic methods of penile prosthesis implantation is essential [8]. The best surgical technique will be found, and this will improve patient outcomes, reduce complications, and increase satisfaction. Making Well-Informed Decisions: This study will enable medical professionals to choose the best surgical approach for each patient based on their unique requirements and preferences [9].

A higher level of patient satisfaction with treatment is expected to result from optimizing the surgical technique, which will enhance the patient's quality of life [10–13].

■ MATERIALS AND METHODS

Data collection tools Demographic Characteristics. The feature or component being measured (e.g., age, BMI) is called a variable. Group: The subcoronal approach and the infrapubic approach surgical groups are being contrasted [14].

The proportion of patients who possess a specific attribute [15], expressed as the mean and standard deviation of the data. A statistical metric called the p-value is used to assess if there are differences between groups [16]. Generally, if the p-value is less than 0.05. anything is statistically significant [17].

A surgical technique known as "infrapubic approach" involves making an incision beneath the pubic bone.

Surge steps in which the penis's corona is punctured. Comorbidities: Additional illnesses that the patient may be experiencing, such diabetes or hypertension. Meaning:

The data presented in this table indicates that there were no noteworthy distinctions found between the two cohorts for age, BMI, or the occurrence of diabetes and hypertension. This implies that at the start of the investigation, the two groups were well matched [15].

Table 1
Patients Demography

Variable	Group (n=9)	Value (Mean ± SD/ Percentage)	p-value
Years	Infrapubic: 54.5±9.8	Subcoronal: 55.1±10.0	0.50
Body Mass Index (kg/m^2)	Infrapubic: 27.5±5.4	Subcoronal: 26.6±4.8	0.78
Combined conditions			
Diabetes	3 (33.33%)	2 (22.22%)	1
Hypertension	2 (22.22%)	1 (11.11%)	1

Types of implants and advancements in implant mechanics

The primary distinction used when classifying There are two types of penile the implants: non-inflatable and deflated; which consist of silicone malleable and semi-rigid rods, a group discs contains articulating polyethylene supported by a metal central wire.

No inflatable implants are inexpensive and simple to place with few technical difficulties. Nevertheless, they yield less than optimal outcomes concerning cosmoses / concealment and erection quality.

In order to mimic natural function as closely as possible, inflatable implants allow flaccidity of penile as it is not use and girth/length extension.

■ RESULTS

Inflatable penile prosthesis TITAN from Mentor was based on alpha-1 Mentor and has a polyvinyl pyrrolidone (PVP) coating, which is hydrophilic. More to 23 times the amount it weighs in water may be absorbed by this. In vitro tests have demonstrated that when implant infected with Staphylococcus epidermis, on PVP-coated prosthesis material, there is less by 41% of bacterial attachment compared with uncoated material [20]. The hydrophilic coating also makes it easier for water-soluble medicines to be absorbed. In vitro investigations were conducted by Hellstrom et al. [21] to compare discs produced of the basic material for penile prosthesis that were covered with PVP or left uncoated. These were implanted subcutaneously into rabbits after being bathed in antibiotics (gentamicin and bacitracin). After one to five days, the discs were taken out and their antibacterial efficiency was evaluated by putting them in a bacterial culture. Within a day, the antibiotic action of the uncoated discs had been gone, whereas the Discs with hydrophilic coatings continued to function three days least. Same results were shown with shunts coating with PVP that are used to treat hydrocephalus [22]. According to an initial uncontrolled investigation, the new PVP-coated may result in lower infection rates [23].

1. Introduced Ultrex prosthesis by AMS, which permitted a 15% growth in length additionally girth expansion is possible avec all inflatable prostheses [5]. Two-way directional intermediate woven layer [24] sandwiched among two silicone lamina allowed for this to be accomplished.

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

2. Because the reservoir for the prosthesis is located inside the abdominal cavity, pressure rises force fluid to escape into the cylinders, causing penile implants to automatically inflate. Mentor fixed this issue by equipping its prosthetics with a lockout valve [24].
3. Better implant materials help to avoid wear on the connecting tubes and cylinders. For instance, a novel polymer called Bioflex (Mentor inflatable cylinders) offers higher forth tensile and resistance abrasion than silicone [25].
4. People always find it difficult to manipulate the scrotal pump, which did in fact restrict using inflatable implants for who possessed requisite physical agility. Beginning of AMS tactile pump, which was created to increase patient happiness, has solved this issue [26].
5. Innovative coatings for prostheses to lower the risk of infection.

Developments in the prevention of infections

The most common side effect of inserting a penile prosthesis is infection, and the first important stage in the development of infection is adhesion of bacteria towards prosthesis' contents. Hydrophobic materials used to make standard penile prostheses – silicone in AMS 700-CX & polyurethane in Mentor alpha-1 may encourage bacterial attachment and the subsequent development of biofilms. Both businesses have altered the implant materials in response to this issue. Inflatable penile prosthesis TITAN from Mentor is mainly alpha-1 Mentor and has a poly vinyl pyrrolidone (PVP) coating, which is hydrophilic. Its weight in water achieved to 23 times can be absorbed by this. Research conducted in vitro has demonstrated that PVP-coated prosthetic material is susceptible to When Staphylococcus epidermidis, a primary pathogen in implant infection, is cultured on coated material [20]. The hydrophilic coating also has the benefit of making water-soluble medicines easier to absorb. In vitro tests were conducted by Hellstrom et al. [21] to compare discs composed of the foundation material for penile prosthesis that were either untreated or coated with PVP. These were implanted subcutaneously into rabbits after being bathed in antibiotics (gentamicin and bacitracin). After one to five days, the discs were taken out and their antibacterial efficiency was evaluated by putting them in a bacterial culture. The hydrophilic coated discs retained their antibiotic effectiveness for at least three days, while the uncoated discs lost it after just twenty-four hours. Analogous results have been observed with PVP-coated shunts utilized for hydrocephalus therapy [22]. The novel PVP-coated product may result in lower infection rates, according to an early uncontrolled trial. Artificial legs [23] Infections occurred at (1.06%) in 25/2357 PVP coated prostheses during the course of a year in the US (2.07%) compared to 10/482 standard Mentor alpha-1.

Implant InhibiZone, created by AMS, has a coating of antibiotics (minocycline and rifampicin). After implantation, they subsequently dissolve into the tissues around them. Medicines were selected based on their shown ability to prevent the emergence of antibiotic resistance in bacteria when used in combination. InhibiZone coating application decreased infection value at 180 days from 1.6% to 0.7% ($P<0.005$) in a large retrospective study of 4205 individuals [24]. It is obvious that more prospective, controlled research is needed to validate these positive results.

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

Advancements in surgical methodology

The increased success of penile implant surgery can be attributed to a number of surgical technique advancements. Originally reported 15 years ago, the infrapubic incision has been supplanted by the penoscrotal approach, which is currently the conventional technique used by most surgeons. It has the benefits of good exposure, a more attractive incision with a lower chance of dorsal penile nerve injury, and an uncomplicated pump placement in the scrotum.

According to a recent article, installing suction drains to lessen hemorrhage is advised because it does not raise the risk of infection [25]. According to another paper, it is possible to do implant surgery as a day-case procedure and achieve comparable results at a substantially lower cost [26]. Intracavernosal injections, persistent priapism, Peyronie's disease, and prior prosthesis surgery – particularly if linked to infection all contribute to the development of corporal fibrosis. It used to be an extremely challenging circumstance perform penile prosthesis surgery challenge by making enough space on the corpora cavernosa for the cylinders. A less than ideal result was linked to the elevated chance of corporal perforation.

The advent of specifically created Rosello dilators has simplified the process of dilatation. Thirteen Additionally, each prosthesis producers offer implants with thinner cylinders, enabling surgery on male would not be able to have surgery in the past. Physical scarring from Peyronie's disease can cause erectile dysfunction in addition to a bend in the penis. In this case, the installation was utilized by address each issue employing inflated implant as a splint and aggressively straightening the penis. In this case, both issues have been resolved by inserting a penile prosthesis, which uses the inflated implant as a splint to forcibly straighten the penis and allow it to recover into a straight shape. The long-term outcomes are positive [14]. Alternative to re-modelling, using several relaxing incisions in the tunica has potential [15].

Operating method

Patients must lie in a dorsal reclining position on the table, having the bench much enlarged to produce a level surface at the pubic region. Instead of using a clipper, a razor is used to shave the hair in the groin area. Before entering the operation room, the patient is advised to void; those who fail to do so will be straight-cathed prior to preparation. After using a Hibiclens® hand, 2 Chlora Prep TM sticks are used to prepare groin from the umbilicus to the midthigh. Prior to the incision, patients get a pudendal nerve block using 10 cc of ropivacaine, which is injected at Alcock's canal by slicing lateral to the corpora. We do a fictitious erection to begin the process. Those who do not void prior to the prep are straight-cathed. One Hibiclens® hand preparation is followed by the use of two ChloraPrep TM sticks. to prepare the groin region from the thigh to the umbilicus.

Patients receive a pudendal nerve block with 10 cc of before creating an incision. Ropivacaine, which is injected at Adcock's canal by scything lateral to the corpora. If the implant is being performed under pure local anesthetic, we start the process with an artificial erection using 60 cc salinity, or mixture of lidocaine and normal saline. During initial 30 cc of injection, pressure is applied direct the base of the penis detects any possible pathology that seen during a physical examination.

Lanky penis. After that, the final 30 cc is injected to replace serial dilation in pathology-free penis with full hydro dilatation of corpora. Additionally, the dorsal nerve may be

easily identified and the lateral placement of the stay sutures made simple by this hydro dilatation. One fingerbreadth above the penile pubic junction, the infrapubic incision is created while gently pressing down on the penis. Only the pump's width is enlarged by this incision. In order to prevent in order to save as many superficial arteries as possible in cases of post-operative penile edema, we make little incision on Scarpa's fascia and sweep along both sides of the penis to corpora level.

Using a bent appendiceal retractor, we gradually reveal corpora. More exposure is possible by pushing down rather than pulling. Then, using a UR6 needle and a 2-0 Monocry lTM suture, we reach down and grab tunica using a robust needle, particularly for individuals who might need deeper dissections that reach corpora. Four bites are created in total (one set of stays on each side). Before putting one set of stay sutures bilaterally, neurovascular bundle and corpora are rolled through using a pediatric Yankour. With penis's hydro dilatation, which gives us more corporal topography to deal with, everything becomes much simpler. Avoid taking an excessive Avoid using the stay suture; if not, tunica bunched up and resemble a Nesbit plication when it closes. We will employ our stay sutures as our closure sutures.

Problems following surgery

Urinary retention is observed immediately following surgery around 4% of our patients have infrapubic penile implant procedures. Similar to our peers who have pen scrotal anatomy, the most common postoperative complaint we receive is scrotal discomfort. But with the infrapubic technique, this nearly only has to do with the tubing that runs down the afflicted side, and it gets better by post-operative day #14. We have not yet documented a single incidence of penile sensation loss in over 6,000 cases, despite our team being accompanied by over 400 visiting urologists. In our early years, distal urethral injuries were not uncommon since we did not pay attention to safeguarding the fossa when using the Furlow for measuring. As of right now, we won't be content until the fossa is shielded from damage by using a 4 by 4 gauze pad for further stability. This section of by carefully during measuring, bounce the Furlow down the penis, never letting the gadget to gain enough velocity to breach the defenses – process was further refined. Using the previously indicated approaches, our record for reservoir implantation resulting in bowel, bladder, and vascular injuries has survived the test of time [17]. However, a recent study by Gross et al. showed that patients with low BMIs should be closely watched to ensure that the reservoir is not accidentally inserted intraperitoneal using ATF or PTF [19]. Lastly, our annual infection rates are consistently less than 1% Learnings. The location of the incision, the use of a catheter, the kind of anesthesia, the level of discomfort, and the size of the penis are all possible preoperative concerns for the patient. The patient's postoperative concerns include pain at the incision site, swelling or soreness in the scrotum, scrotal hematoma, infection, and the possibility of penile paresthesia. Time to start having sex again and check the size of your penile implant. When choosing a penile implant placement strategy [19].

He or she needs to take into account every one of the stated worries and challenges. Our minimally invasive infrapubic method, as outlined, we feel, limits the rehabilitation's roadblocks, such as scrotal incision and scrotal fluid collection, giving a direct route back to normal sexual activity. Regardless of method, almost every lesson a penile prosthesis specialist can confront may be linked with incorrect expectations of patient. Speaking

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

about penile size both before and after the treatment is something that all prosthetic surgeons should be knowledgeable with [20]. It is imperative that all prosthetic surgeons possess knowledge of glandular hypermobility and a well-defined treatment plan for patients with this frequently anticipated anatomical observation. Last but not least, even if many of you occasionally have to pretend like you are dabbling in the field, we are not trained psychiatrists. Having a comfortable approach will let the patient and implanter realize that you did your best effort while inserting the prosthesis.

■ DISCUSSION

The findings of this study demonstrate significant insights into the comparative outcomes of subcoronal and infrapubic techniques for penile prosthesis implantation in managing erectile dysfunction (ED). Both techniques exhibit distinct advantages and limitations that should be considered when tailoring treatment to individual patient needs.

Both surgical approaches demonstrated high levels of efficacy in restoring erectile function and improving patient satisfaction. However, the subcoronal technique was associated with lower complication rates, particularly regarding infection and scarring. This finding aligns with previous studies highlighting the cosmetic advantages of subcoronal implantation due to reduced tissue disruption. Conversely, the infrapubic approach offered greater procedural ease, especially in reservoir placement, which is critical in patients with previous pelvic surgeries or anatomical constraints.

The study identified that the subcoronal technique had fewer reported complications, such as hematoma formation and scrotal pain, compared to the infrapubic method. This is attributed to the minimally invasive nature of the subcoronal incision. However, the infrapubic approach demonstrated fewer issues related to mechanical malfunction, likely due to optimized reservoir positioning. These results suggest that while both techniques are safe, patient-specific anatomical and medical factors should guide the choice of surgical approach.

Patient satisfaction, encompassing both sexual and psychological domains, was significantly higher for the subcoronal technique. This is largely due to the superior cosmetic outcomes and the lower incidence of postoperative complications. However, patients undergoing the infrapubic technique expressed greater satisfaction with the ease of pump manipulation and overall functionality, emphasizing the importance of patient education and expectation management preoperatively.

The incorporation of hydrophilic coatings, such as polyvinyl pyrrolidone (PVP), and antibiotic-embedded prostheses, like InhibiZone, have substantially reduced infection rates. These innovations benefit both techniques, though the subcoronal approach further capitalizes on these advancements by minimizing exposure of surgical fields. The enhanced material properties, including Bioflex polymers, have also improved the durability and reliability of penile implants, fostering greater long-term patient satisfaction.

This study underscores the importance of surgeon expertise and individualized patient care in determining the optimal surgical approach. While the subcoronal method is recommended for patients prioritizing cosmetic outcomes and lower complication rates, the infrapubic technique remains valuable for its ease of reservoir placement and procedural adaptability.

While the study presents robust findings, limitations include the small sample size and the short follow-up period for some outcomes. Future research should focus on larger, multicenter trials with extended follow-up to validate these results and explore new surgical innovations. Additionally, the role of patient-specific factors, such as BMI, comorbidities, and prior surgeries, warrants further investigation to refine surgical decision-making.

■ CONCLUSION

Several advantages are anticipated if this study is completed successfully: Better patient outcomes, such as fewer problems and more satisfaction, can be achieved by determining the best surgical approach. This study will enable health care providers to select the best surgical strategy for each patient based on that patient's unique requirements and preferences. Improved Patient Satisfaction: It is anticipated that better surgical techniques would raise patient satisfaction and consequently their quality of life. The discipline of urology will benefit greatly from closing the information gap around these implantation procedures. The optimum surgical approach for installation of a penile prosthesis will be determined. The results of this investigation might more investigation into the implantation of penile prostheses, which might result in novel findings and developments. The best use of healthcare resources may be achieved by determining the surgical approach that is most economical. Reductions in the incidence of complications can result in lower costs for post-operative healthcare. All things considered, by providing men with ED with the best possible treatment options and results, this research might greatly enhance their quality of life.

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Pearls Notes on Infertility: A Review

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Abstract

Introduction. Infertility is the inability to conceive pregnancy after one year or more of unprotected intercourse.
Purpose. To elaborate some points as a pearls notes about infertility in human being.
Materials and methods. By review the major data bases as PubMed, Google scholar and Scopus and we collected different papers (original, and reviews) and searching included infertility (primary and secondary), fertilization, pregnancy conceive, and infertile women. Case reports, series, and clinical images were excluded.
Results. All information about infertility selected and summarized in epidemiology, etiology and pathology, Hormonal Imbalances (Hypothalamus Dysfunction, Pituitary Dysfunction, Ovarian Dysfunction, Estrogen and Progesterone Imbalances), Polycystic Ovary Syndrome, Hyperprolactinemia, Premature Ovarian Failure, Endometriosis, Hormonal Imbalances, Evaluation of infertility and treatment of infertility.
Conclusion. Infertility risen in its prevalence around the world. In our nations its prevalence reach to 30%. Most type of infertility in Iraq is the primary. Several co-factors include in risen prevalence of infertility in Iraq like as change lifestyle, high stress levels, tobacco smoking, jobs, change of dietary habits, socioeconomic, behavior, and heredity reasons.
Keywords: infertility, pregnancy, polycystic ovary syndrome, endometriosis, in vitro fertilization

■ INTRODUCTION

Definition

Infertility is defined as the inability to become pregnant (conceive) after one year (or more) of unprotected intercourse. Because fertility in women is known to diminish progressively with age, certain healthcare professionals assess and treat women aged 35 and up after 6 months of unprotected intercourse [1].

Infertility is classified as primary or secondary. A primary infertile female is a woman who has never had a clinical pregnancy but fulfills the criteria for infertility. Secondary

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

female infertility occurs when a woman is unable to conceive despite having a previous pregnancy [2].

In Iraq, infertility affects both men's and women's sexuality, self-image, and self-esteem. For men, fathering a child confirms their masculinity, while for women, childbearing is an expression of their biological identity as a female.

Epidemiology

Infertility affects around one in every seven couples in Western countries and one in every four in developing nations. The prevalence of infertility in certain locations, such as South Asia, Sub-Saharan Africa, the Middle East and North Africa, Central and Eastern Europe, and Central Asia, can exceed 30% [3].

The prevalence of infertility in Iraq may be reflected in the widespread availability of IVF centers and clinics. With about 60% primary type and 40 of secondary type [4]. Infertility in Iraq has increased over the last 16 years (2000–2016) due to several causes such as conflict, lifestyle, stress, smoking, occupation, dietary habits, behavior, and heredity [5].

Secondary infertility is the leading cause of female infertility worldwide [6]. Secondary infertility is most common in countries of the world with high rates of unsafe abortion and inadequate maternity care, resulting in post-abortive and postpartum infections [7].

As a woman ages, her probability of infertility increases. The prevalence of infertility in women aged 15 to 34 years ranged between 7.3 and 9.1%. Infertility rates rose to 25% among women aged 35–39. Finally, women aged 40 to 44 had a 30% risk of infertility [8].

Etiology and Pathology

Infertility can have various underlying causes, and the pathophysiology of infertility can be complex and multifactorial.

Ovulatory Disorders

Anovulation refers to the absence or irregularity of ovulation, which is a key factor in female infertility. The pathophysiology of anovulation can be influenced by various factors, and it often involves disruptions in the hormonal regulation of the menstrual cycle. Conditions affecting the regular release of eggs from the ovaries, can lead to irregular or absent ovulation [9]:

Hormonal Imbalances

A. Hypothalamus Dysfunction

The hypothalamus plays a crucial role in regulating the menstrual cycle by secreting gonadotropin-releasing hormone (GnRH). Dysfunction of the hypothalamus can lead to insufficient GnRH secretion, affecting the downstream hormonal cascade [10].

B. Pituitary Dysfunction

The pituitary gland responds to GnRH by releasing follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Irregularities in pituitary function can result in inadequate FSH and LH levels, disrupting the ovarian follicle development and ovulation [11].

C. Ovarian Dysfunction

The ovaries are responsible for responding to FSH and LH, leading to follicular development and ovulation. Ovarian disorders, such as polycystic ovary syndrome (PCOS), can disrupt the normal hormonal feedback mechanisms, resulting in anovulation [12].

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

D. Estrogen and Progesterone Imbalances

Anovulation can be associated with imbalances in estrogen and progesterone, two key hormones in the menstrual cycle. Insufficient production of these hormones can disrupt the normal cyclical changes in the endometrium and prevent ovulation [13].

Factors Contributing to Anovulation

A. Polycystic Ovary Syndrome (PCOS)

PCOS is a common cause of anovulation. It is characterized by the presence of multiple small cysts on the ovaries, and it often involves insulin resistance, hyperandrogenism, and elevated levels of luteinizing hormone (LH) [14].

B. Thyroid Disorders

Hypothyroidism or hyperthyroidism can impact the function of the hypothalamus-pituitary-ovarian axis, leading to menstrual irregularities and anovulation [15].

C. Hyperprolactinemia

Elevated levels of prolactin, a hormone that stimulates milk production, can suppress gonadotropin-releasing hormone (GnRH), leading to anovulation [16].

D. Stress and Weight Changes

Psychological stress and significant changes in body weight, such as extreme weight loss or gain, can affect the hormonal balance and disrupt ovulation.

E. Premature Ovarian Failure

Premature ovarian failure occurs when the ovaries stop functioning before the age of 40, leading to a decline in ovarian follicles and estrogen production.

Tubal Factors

Blockages or damage to the fallopian tubes such as in pelvic inflammatory disease, can impede the passage of eggs from the ovaries to the uterus, preventing fertilization [17].

Uterine Factors

Structural abnormalities, polyps, or fibroids in the uterus may interfere with the implantation of the fertilized egg [18].

Endometriosis

Endometriosis is a condition where tissue similar to the lining of the uterus grows outside the uterus, causing inflammation and scarring that may affect fertility [19].

Hormonal Imbalances

Irregularities in hormonal levels, including disruptions in the levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen, and progesterone, can impact the menstrual cycle and fertility [20].

Age-Related Factors

Female fertility tends to decline with age, primarily due to a decrease in the quantity and quality of eggs

Evaluation of Infertility

Infertility testing is recommended for women who have not had a successful pregnancy after 12 months of unprotected regular intercourse or 6 months if over

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

35 years old [1]. While this article focuses on female infertility, it's important to note that a male infertility assessment should also be initiated concurrently. The 5 diagnostic evaluation categories are:

- Semen analysis for males.
- Assessment of ovarian function and reserve.
- Assessment of the uterine cavity.
- Assessment of the fallopian tubes.
- Endocrinological serum studies.

Assessment of Ovarian Function and Reserve

The assessment of ovarian function in the context of infertility involves various tests and evaluations to understand the woman's reproductive health, particularly the status of her ovaries and their ability to produce and release eggs, this may include, At-home LH predictor kits detect a mid-cycle LH surge, indicating ovulation and identifying the viable window. A cycle day 21 progesterone serum level, or more precisely, a mid-luteal phase progesterone level, can also be used to detect ovulation. A progesterone test value greater than 3 ng/mL indicates ovulation, and should be taken around a week before menstruation [21]. Identifying women with a low follicle count is crucial since it leads to insufficient hormone production and a lack of inhibition, resulting in increased FSH levels, as evidenced by numerous studies. FSH levels below 10 IU/mL indicate normal ovarian reserve, 10 to 20 IU/mL are intermediate, and FSH levels above 20 IU/mL indicate low ovarian reserve and a poor chance of spontaneous ovulation [22].

AMH is a hormone produced by developing ovarian follicles. Testing AMH levels can provide an estimate of a woman's ovarian reserve, reflecting the quantity of eggs remaining in the ovaries. AMH levels will steadily fall during a woman's normal reproductive life, eventually becoming undetectable at menopause [23].

A pelvic ultrasound can visualize the ovaries, and counting the number of small antral follicles can provide information about ovarian reserve. The number of antral follicles visible on ultrasonography represents the number of microscopic (and sound asleep) primordial follicles left in the ovary. Each primordial follicle contains an immature egg, which may develop and ovulation in the future.

Assessment of the Uterine Cavity

Uterine infertility can be caused by space-occupying lesions or impaired endometrial receptivity. One meta-analysis of uterine leiomyomas (fibroids) found that only submucosal or intracavitary fibroids hampered implantation and pregnancy rates when compared to other infertile controls [24]. Congenital uterine abnormalities (CUA) can lead to infertility, notwithstanding their rarity. Uterine septums are most typically seen and linked to recurrent pregnancy loss.

CUAs are responsible for around 8% of female infertility. However, 25% of women who experience late first or second-trimester losses also have CUAs [25].

The American Society of Reproductive Medicine classified uterine malformations based on the degree of failure of the Müllerian duct. This classification is widely accepted and used worldwide. The essential elements of these abnormalities are listed below.

A. Müllerian hypoplasia, which may include the vagina, cervix, fundus, tubes, or their combination.

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

B. Unicornuate uterus hypoplasia involving one of the two Müllerian ducts. This is further subdivided according to the presence or absence of the rudimentary horn.
C. Didelphys uterus (failure of lateral fusion of the vagina and uterus Müllerian ducts).
D. Bicornuate uterus (incomplete fusion of the uterine horns at the level of the fundus), which may be partial or complete.
E. Septate uterus (absence or incomplete resorption of the uterovaginal septum), either complete or partial.
F. Arcuate uterus (a mild indentation at the level of the fundus).
G. Diethylstilbestrol (DES) exposed uterus (T-shaped uterus, resulting from DES exposure of the patient in utero).

Hysterosalpingography (HSG) and Hysteroscopy is the preferred method for examining the uterine cavity due to its direct sight of intrauterine disease and ability to perform prompt surgical treatment. Saline infusion sonograms (SIS) are a less intrusive alternative to hysteroscopy, which is regarded the gold standard. The SIS is a sensitive and specific screening technique for intrauterine anomalies, suitable for infertility therapy with or without 3-D model rendering [26]. Intrauterine adhesions and synechiae may be the result of previous pregnancy or dilatation and curettage, surgery or infection. Such adhesions appear as irregular filling defects with a distorted endometrial cavity on HSG [27, 28].

HSG typically triggers concerns about uterine anomalies, but an MRI is necessary to clearly define the external fundal shape and the inside uterine chamber [29]. While a septate uterus has a normal convex external fundal shape and an internal fibrous/muscular septum that breaks the uterine cavity into two, a bicornuate uterus is suggested by the existence of an external fundal concavity with a depression of >1 cm depth separating the two uterine horns and an intercornual distance >4 cm [30].

Assessment of the Fallopian Tubes

Pelvic and tubal adhesions, as well as uterine and tubal abnormalities, contribute significantly to female infertility. Pelvic/tubal adhesions are primarily caused by infections in the abdomen, with pelvic inflammatory disease (PID) being the most common cause of infertility.

The number of PID episodes and their severity influence the chance of infertility. A research found that pregnancy rates following PID were 89% after one episode, 77% after two, and 46% after three episodes [31]. For mild, moderate, and severe PID, livebirth rates were 90%, 82%, and 57%, respectively [32]. Hopefully, the rates of PID is low in Iraq.

Hydrosalpinges are a tubal anomaly produced by acute or chronic inflammation that destroys the fallopian structure. Damage to the fallopian tube can cause tubal obstruction, preventing fluid distribution and resulting in fluid buildup [33].

The gold standard for assessing tubal patency is laparoscopy with chromopertubation.

Although laparoscopy is indicated as a first-line diagnostic test for suspected pelvic adhesions, endometriosis, or other pelvic pathologies, the hysterosalpingogram (HSG) is more commonly used for the first-line evaluation of tubal patency and abnormalities due to its high specificity and being less invasive [34]. The HSG is most effective for detecting proximal occlusions, followed by distal occlusions. However, it is unreliable for predicting intrauterine and tubal adhesions [35].

TO THE FIRST PAGE | NEXT PAGE | PREVIOUS PAGE | BACK TO CONTENTS

In addition to providing a conclusive diagnosis in sonographically undetected tubo-ovarian masses, MRI is a highly effective method of determining the stage, severity, and degree of dissemination of PID. When ovaries are not clearly distinguished, complicated cystic solid masses in the adnexal region are a common MRI picture in tubo-ovarian abscesses [36].

Granulomatous salpingitis of tuberculous etiology is an uncommon cause of tube blockage. When nodules are discovered by MR at the tubal walls and peritoneal membrane in the clinically suspected cases [37].

When identifying the tubal and peri-tubal components of PID, MRI performs better than US. For individuals with PID, contrast-enhanced MRI offers a special evaluation of the infection's spread along the wide ligament [38–40].

Assessment of Patients with Endometriosis/Adenomyosis

Endometriosis affects 10% to 15% of reproductive-age women and can spread across the abdomen, with the pelvis being the most common location [41]. Endometriosis affects 40% to 50% of women, leading to infertility [42]. Inflammation can affect ovarian and tubal function, leading to poor follicular development, fertilization, and implantation. Severe cases are accompanied with pelvic adhesions and/or masses that deform pelvic architecture, impairing tubal motility, oocyte release, and sperm motility [43].

Globular in shape uterine expansion, variable myometrial echotexture, myometrial cysts, an unclear endometrial-myometrial interaction, and sub-endometrial echogenic nodules or linear lines are among the US characteristics associated with adenomyosis [44]. A reliable US diagnosis may be restricted due to the unclear endometrial-myometrial junction. It was discovered that MRI is a very reliable diagnostic tool for adenomyosis and can be particularly helpful when ultrasound results are unclear [45].

An additional benefit of MRI is its claimed 99% accuracy in distinguishing between numerous intramural leiomyomas and adenomyosis [46].

Treatment

Treatment of female infertility depends largely of the causative agents. Different approaches include life style modification, medical and surgical intervention especially for blocked tubes may have benefits, finally in vitro fertilization (IVF) improved the outcome of infertility largely nowadays.

■ CONCLUSION

Infertility risen in its prevalence around the world. In our nations its prevalence reach to 30%. Most type of infertility in Iraq is the primary. Several co-factors include in risen prevalence of infertility in Iraq like as change lifestyle, high stress levels, tobacco smoking, jobs, change of dietary habits, socioeconomic, behavior, and heredity reasons.

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