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Bouquet in a Niche. Detail. By the Southern Netherlandish artist Osias Beert the Elder (1580–1624).
In his floral still lifes, he often depicted a vase of flowers in a small niche.
Butterflies in his works symbolized salvation and resurrection. He also played an important role
in the development of the "breakfast still life" genre as an independent movement in Northern European painting.
The artist did not date his paintings and very rarely signed them.

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Effect of Adding Honey Bee on Sperm Functions of Patients with Teratozoospemia During Freezing

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Abstract

Introduction. Infertility is identified after unprotected sexual activity without conception. The specific composition of honey bee serves as nutrient of sperm and antioxidant against free radical during freezing.
Purpose. To explore the consequence of adding honey bee in different concentrations to freezing solutions on count sperm, total motility%, normal morphology% and vitality% for Teratozoospemia.
Materials and methods. This research is achieved in fertility Center at Al-Sadr Medical City in Al-Najaf, Iraq. The assortment age of Teratozoosperma and control ranged from 25 to 47 years. The study included 120 individuals (70 Teratozoospermia and 50 control). After taking semen samples from both Teratozoosoermia and control and completed liquefying them, the semen was analyzed before freezing and measured count sperm, Total motility%, normal morphology% and vitality%. Then added honey bee at concentration 15% and 20% to solution of freezing (Sperm freeze medium and 15% glyceror). Each semen sample divided into three groups depending on honey bee concentration are G1: without honey bee, G2: contain 15% concentration and G3: contain 20% concentration, then semen sample preserved using freezing in liquid nitrogen.
Results. The outcomes designated non-significant different ($p<0.05$) in count sperm, while noted total mortality%, normal morphology and vitality% were decreased significantly($p<0.05$) in teratozoospermia paralleled with control men. The current study indicated the total sperm count, total motility%, normal morphology% and vitality% stood reduced considerably ($p<0.05$) after thawing in all groups of teratozoospermia (G1, G2 and G3) comparing with before Freezing.
Conclusion. Adding honey bee at certain concentration (15% and 20%) to the freezing solution that will reduce the harmful effects of freezing in liquid nitrogen on frozen sperm.
Keywords: freezing, honey bee, teratozoospermia, total motility%, vitality%

■ INTRODUCTION

After at least a year of routine, unprotected sexual activity without conception, infertility is identified [1]. Approximately 10% of the population suffers from this rather common ailment. There are two types of infertility: primary, in which there has never been a pregnancy, and secondary, in which a pregnancy has occurred, regardless of its outcome. Primary and secondary infertility affect roughly 67–71 percent and 29–33 percent of patients, respectively [2]. The largest known cause of infertility in humans is defective sperm function, which is present in half of these cases due to a male factor [3]. Male infertility in the majority of the species under study have long been linked to sperm abnormalities. These anomalies can range from morphological flaws that are obvious during a clinical examination to more subtle flaws. Generally speaking, sperm structure can have a significant impact on the outcome of fertilization and pregnancy [4]. Defective sperm structure can be caused by genetic, environmental, or a combination of factors. There is a growing list of sperm structural defects that are thought to have a genetic origin, even though environmental causes are thought to be the most prevalent. Non-systematic or nonspecific sperm sperm defects and systematic sperm defects are the two primary types of ultrastructural sperm anomalies [5].

Rendering to the WHO strategies, nearly 15% of men suffering from infertility cannot be identified with a definite reason for their problem. This circumstance in men is categorized as unexplained infertility and is distinct as couples in whom repetitive semen examination is within normal standards [6]. There are significant amounts of statistics that link oxidative stress as a significant and possible source of idiopathic male infertility [7]. Other reasons including lifestyle and environmental issues are assumed to perform an increasing part. Teratozoospermia is defined as the decrease of the percentage of normal sperm morphology)less than 30%) [8]. Spermatozoa morphology is crucial for early embryonic development and successful fertilization in assisted reproductive techniques [9]. As a standard procedure in assisted reproductive technology (ART), freezing sperm in liquid nitrogen (–96 °C) is one of the greatest widely used and accepted methods to reservation a man's fertility possible. This strategy becomes extremely important when man fertility requirements to be maintained prior to cytotoxic chemo-therapy, radiation therapy, or certain surgical procedures [10]. With a well-established role and growing significance in human assisted reproduction from the early stages of this technology's development due to a variety of clinical, ethical, and legal considerations, the ability to freeze biological cells and tissue in liquid nitrogen (–196 °C) while maintaining their structure and function is important in many areas of biology and clinical medicine [11].

The freezing process is a crucial tool for the long-term storage of viable sperm. Patients with prostate cancer, testicular tumors, and infertile men can also use it to help with reproduction [12].

When sperm are frozen in liquid nitrogen (–196 °C), their structure and function may be negatively altered [13]. Numerous damage processes, including cold shock, ice crystal formation, cell dehydration, free radicals, oxidative stress, and osmotic shock, have been extensively documented to occur during the freezing and thawing of human spermatozoa [14]. Honey has a natural sweetness. It is created by honey bees as of plant nectar, plant excretions, or plant-sucking insects' excretions on plant portions. The bees then gather these supplies, transform them through combining them with their own substances, dehydrate, deposit, store, and leave in honeycombs to ripen and mature [15]. Honey bee

collected from sugars, vitamins, minerals, amino acids, organic acids, enzymes, vitamins, flavor compounds and many antioxidant enzyme (catalase, glucose oxidase, peroxidase) [16]. The specific composition of honey bee serves as nutrient of sperm and antioxidant against free radical during freezing.

■ MATERIALS AND METHODS

Subjects and location of study

The current research achieved in fertility Center at Al-Sadr Medical City in Al-Najaf, Iraq. The study including 120 men (70 patients with Teratozoospermia and 50 control). The age of Teratozoosperma and control ranged from 25 to 47 years.

Sample's collection and analysis

Samples from patients and control, were collected in sterile containers and placed in an incubator for 30 minutes. The following criteria (total sperm count, total motility%, normal morphology% and vitality%) were measured based on [17, 18].

Assessment of total sperm count

The sperm count is the total number of sperm in an ejaculate. The sperm concentration was multiplied by the volume of semen to determine the total number of sperm. The lower reference limit for the total number of sperm is 40×10^6 spermatozoa per ejaculate. The mean number of sperm in five high power fields (HPF) with an objective lens magnification of 40X was used to calculate the sperm concentration. The total number of sperm was determined by [17]:

Total sperm count (million/ejaculate) = sperm concentration × volume.

Valuation of total motility%

Sperm motility was determined by examining the prepared slide. Count and motility should be assessed within an hour. It was examined under the microscope right away to avoid the results being impacted by the heat from the light source. Five randomly chosen fields were counted for the quantity of motile sperm away from the cover slip edge. A minimum of one hundred sperm cells were counted [19]:

$$\text{Total sperms motility} = \frac{\text{No. of motile sperms}}{\text{Total No. of sperms}} \times 10.$$

Calculation of normal sperm morphology

It is challenging to assess human spermatozoa due to their variable morphology [20]. Thirty percent is the range of normal values. The similar slides that were prepared for sperm motility stayed used to examine morphologically normal sperms (MNS). The number of spermatozoa was at least 100. The following formula was used to determine the MNS percentage [17]:

$$\text{Normal sperm (\%)} = \frac{\text{No. normal sperms}}{\text{Total No. sperms (normal and abnormal)}} \times 100.$$

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Calculation of vitality%

Sperm vitality was evaluated using a combination of Nigrosin (0.1 percent) and Eosin Y (0.5 percent) staining, 50 µl of semen was combined with an equivalent volume of eosin-nigrosin suspension. After creating a smear on a glass slide, they were left to air dry. While live sperms maintained the integrity of their cellular membranes, preventing the stain from penetrating cells, dead perms were stained with Eosin. The background stained by nigrosin. 200 sperm in all were evaluated using a high-resolution (100X) objective lens of a light microscope whereas submerged in oil. White spermatozoa that stayed unspotted were considered as "live", while any pink or red spermatozoa considered as "dead" [18, 21].

Procedure of freezing and thawing

Before freezing, each semen sample was split into three groups and put in a cryovail; G1 contained 0.7 ml of semen and 1 mL of freezing solution (Sperm freeze medium and 15% glyceror) only, G2 was 0.7 mL semen with1Ml freezing solution contains 15% honey bee, G3 was 0.7 mL semen with 1mL freezing solution contains 20% honey bee. Then all sample groups were preserved by freezing in liquid nitrogen (LN2) temperature (–196 °C). For four months, following four months of freezing in liquid nitrogen, each semen sample was thawed [22, 23].

All tests of semen (count sperm, total motality%, normal morphology and vitiality%) that were performed pre-freezing will be repeated on all groups after thawing

Statistical analysis

Analysis of data was performed by using SPSS (Version23). ANOVA and student t-test stayed used to comparison between the groups, then LSD practical. A significance difference at $p<0.05$ [24].

■ RESULTS

Comparison between teratozoospermia and control before freezing

The findings revealed a non-significant alteration ($p<0.05$) in the number of sperm in Teratozoospermia compared to control, but a important reduction ($p<0.05$) in the percentages of total motility, normal morphology%, and vitality% (Table 1).

Table 1
Sperm parameters of Teratozoospermia and control before freezing

Sperm Parameters	Groups	
	Teratozoospermia	Control
Total sperm count (10^6/ejaculate)	49±2.70a	50±1.21a
Total motility (%)	54.62±1.77a	67.43±3.45b
Normal morphology (%)	26.15±1.32a	72.36±2.26b
Vitality (%)	74.53±4.11a	86.18±3.45b

Note: means with different letter are considerably various at $p<0.05$.

Comparison of teratozoospermia between before freezing and after thawing

Teratozoospermia results showed that all groups (G1: no honey bee, G2: 15% honey bee, and G3: 20% honey bee) had significantly lower total sperm count, total motility, normal morphology, and vitality after thawing than before freezing, as shown in table 2.

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Table 2
Sperm parameters of teratozoospermia before freezing and after thawing

Sperm parameters	Before freezing	After thawing			LSD
		G1 (without honey bee)	G2 (15% honey bee)	G3 (20% honey bee)	
Total sperm count (10^6/ejaculate)	49±2.70 a	19.27±1.23b	18.73±1.89c	19.16±1.02d	2.361
Total motility (%)	54.62±1.77a	21.05±3.16b	38.17±2.91c	37.59±2.35d	2.078
Normal morphology (%)	26.15±1.32a	14.13±2.51b	20.53±1.15c	20.61±2.05d	1.726
Vitality (%)	74.53±4.11a	29.56±4.12b	43.17±3.17c	42.68±3.01d	1.561

Note: means with different letters are significantly different when comparing between each group after thawing with before freezing.

Comparison between groups of teratozoospermia (G1: Without honey bee, G2: 15% honey bee and G3: 20% honey bee) after thawing

When compared between groups of teratozoospermia after defrosting, the exhibited an important increase ($p<0.05$) in Total motility (%), Normal morphology (%) and Vitality (%) in both G2: (15% honey bee) and G3 (20% honey bee) compared with G1: Without honey bee, while noted non-significant difference in total sperm count when compared between G1, G2 and G3. Whereas the outcomes recoded non-significant alteration ($p<0.05$) in all sperm parameters when comparing between G3 (20% honey bee) and G2 (15% honey bee), as in table 3.

Table 3
Comparison of sperm parameters between groups of teratozoospermia after thawing

Sperm parameters	Groups			LSD
	G1 (without honey bee)	G2 (15%honey bee)	G3 (20%honey bee)	
Total sperm count (10^6/ejaculate)	19.27±1.23a	18.73±1.89 a	19.16±1.02a	2.043
Total motility (%)	21.05±3.16a	38.17±2.91 b	37.59±2.35b	1.237
Normal morphology (%)	14.13±2.51a	20.53±1.15 b	20.61±2.05b	2.163
Vitality (%)	29.56±4.12a	43.17±3.17 b	42.68±3.01b	1.057

Note: means with different letters are considerably diverse when comparing between groups of teratozoospermia after thawing.

■ DISCUSSION

The condition of unusual sperm shape is called teratozoospermia, it is one of the main factors causing infertility. A defect in the sperm's head, neck, or tail causes some sperm disorders that alter their shape and reduce their chances of fertilizing eggs, even though some variation in the sperm morphology is fine, problems arise when this count goes way beyond the normal levels. This can result in male infertility [25].

The outcomes of this research showed an important decline ($p<0.05$) in total motility%, normal morphology% and vitality%, in Teratozoospermia compared with control men. Most of the time, the causes of teratozoospermia are unknown. But lifestyle and habits (smoking, exposure to toxins, etc. can result in poor morphology as well. Another disorder that is frequently linked to diminished normal forms morphology is varicocele, where veins enlarge within the scrotum, leading to higher testicular temperature. This increased

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temperature may result in abnormal sperm morphology, Testosterone and other sex hormones can affect sperm development and shape. Teratozoospermia can be the result of mutations or gene abnormalities linked to sperm formation [26, 27]. Because this condition is typically asymptomatic, some men who have it do not show any symptoms. Nonetheless, the primary sign of teratozoospermia-related infertility problems in a pair is infertility following frequent, unprotected sexual activity [28].

From this study we found an important decline in sperm parameters (Whole sperm count, Whole motility%, normal morphology% and vitality%) in all groups of Teratozoospermia after freezing compared with group before freezing. These results may be due to freezing and thawing potentially induces sperm damage. Freezing can lead to formation intracellular ice crystals formation injuring sperm plasma membrane, enhance the generation of reactive oxygen species (ROS) and free radicals, which reduce membrane fluidity, increase DNA fragmentation, and impair spermatozoa's capacity to fertilize. Increases in ROS production during freezing may be one of the contributing factors to sperm viability declines following thawing, as ROS levels rise during thawing and superoxide anion production rises as a result of decreased superoxide dismutase activity [29, 30]. Lipid peroxidation of the sperm outer membrane, which results in a decrease in sperm motility and an increase in chromatin and plasma membrane damage, is linked to oxidative stress [31]. A rapid variation in osmolarity often happens throughout freezing and thawing which sources deformities of membranous structures associated with flagellum damage Which leads to increased deformities in the shape of sperm [32].

When compared between the groups of teratozoospermia after thawing showed a significant increase in sperm parameters (total motility%, normal morphology% and vitality%) of G1 (without honey bee) compared with G2 (15%honey bee) and G3 (20%honey bee) these results may be due to Sperm lipid membranes contain a particularly high percentage of poly-unsaturated fatty acids. These polyunsaturated fatty acids (PUFAs) give the plasma membrane the fluidity needed to participate in membrane effusion events associated with fertilization. However, PUFAs oxidized during freezing lead lipid peroxidation [33, 34].

Honey bee in both concentration act as antioxidants that works against reactive oxygen species and protect sperms from the effect of oxidative stress during freezing and thawing because, the honey bee comprises both enzymatic antioxidants (catalase, oxidase, glucose peroxidase) and non-enzymatic antioxidants (ascorbic acid, carotenoids, α-tocopherol, amino acids, flavonoids, proteins, and phenolic acids) [35, 36]. These antioxidant properties of honey may help in reducing free radicals and reactive oxygen species (ROS) which effect on sperm during freezing and thawing.

Honey bee contain many nutrients such as vitamins (A, C, E) and Sugars make up about 79.6% of honey, with levulose and dextrose accounting for 38.2% and 31.3% of the total sugar content, respectively. Whereas the residual percentage are sucrose and maltose [36, 37].

Sugars in honey bee may play an important role during sperm freezing because the sugars combine with lipids present in the sperm membrane, and thus will reduce the damage of freezing to the membrane [38]. Sugars are also the source of energy that provides sperm during their freezing period [39].

Phenolic compounds in honey bee may protect frozen sperm from damage of oxidative stress by directly neutralizing reactive oxidants through its electron donating capability, which leads to oxidation of cell components (lipid, protein and nucleic acid). Since the phenolic compounds in honey bees work to neutralize free radicals, they will prime to a reduction in lipid peroxidation and an increase in the activity of antioxidant enzymes [40, 41].

■ CONCLUSION

The freezing of semen in liquid nitrogen (–196 °C) has numerous negative effects on frozen sperm of teratozoospermia. Additionally, we indicate that adding honey bee at certain concentration (15% and 20%) to the freezing solution that will reduce the harmful effects of freezing in liquid nitrogen on frozen sperm.

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The Role of Some Serum Hormones Level and USP9Y Gene Polymorphisms in the Incidence of Sever Oligozoospermia and Azoospermia in Infertility Patient

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Abstract

Introduction. Male infertility is a multifactorial condition influenced by hormonal and genetic factors.
Purpose. This study investigated the relationship between male infertility and polymorphisms in the USP9Y gene, as well as key reproductive hormones.
Materials and methods. The study included 150 individuals, 50 as a control group, and 100 patients with Azoospermia and sever Oligospermia, infertile Iraqi males, whose ages ranged from 20 to 45 years, at AL-Nasiriyah Teaching Hospital Infertility unite in Thi-Qar Province, South of Iraq. The USP9Y polymorphisms detected by HRM-qPCR while, the hormones were evaluated by ELISA.
Results. The current study recorded that the mean level of LH, E2 and Prolactin hormones in Azoospermia and Oligospermia which was significantly ($P \leq 0.01$) higher than that of the control mean patients compared to controls, while the mean level of FSH and AMH hormone were non-significantly associated with infertility while Testosterone hormone in Azoospermia and Oligospermia was significantly ($P \leq 0.01$) lower than that of control group. As for USP9Y gene the first SNP (rs112694338) revels that the mutant allele T which is more distributed in Azoospermia mostly and Oligospermia as well consider to be the risk allele. While the second SNP (rs3212291) the polymorphism of this SNP plays a role in the genetic susceptibility to oligospermia.
Conclusion. This study investigated there were hormonal imbalance in the patients than control group, also noted the polymorphisms in the USP9Y gene is related to Sever Oligozoospermia and Azoospermia.
Keywords: USP9Y gene, male infertility, hormones level, sperm parameters, semen

■ INTRODUCTION

Male infertility is defined as the inability of a male to make a fertile female pregnant, also for a minimum of at least one year of unprotected intercourse. The male factor is substantially contributory in about 50% of all cases of infertility. As male and female causes often co-exist, it is important that both partners are investigated for infertility and managed together [1, 2]. Male infertility is commonly due to deficiencies in the semen, and semen quality is used as a surrogate measure of male fecundity. Males with sperm parameters below the WHO normal values are considered to have male factor infertility [3]. As high as 90% of male infertility problems are related to count and there is a positive association between the abnormal semen parameters and sperm count. The problem with sperm count, motility, and morphology stems from disarray in control mechanism, including pre- testicular, testicular, and post-testicular factors [4].

The causes of male infertility may be acquired, congenital or sometimes idiopathic. All these factors adversely affect the spermatogenesis process as well as they impart serious threats to male genital organs thus resulting in infertility [5]. Some of these causes include smoking, alcohol intake, drugs, obesity, past or present testicular infections, exposure to environmental toxins, exposure of the testicles to excessive heat, hormonal disorders, testicular trauma, ejaculatory/erectile disorders, viral infections and genetic disorders which also considered a risk factor in male infertility [6, 7].

Hormones control the entire male reproductive system. These are chemicals that stimulate or regulate cell or organ activity. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone are the primary hormones involved in the functioning of the male reproductive system. The pituitary gland produces FSH and LH. It is located at the base of the brain and is in charge of many bodily functions. FSH is required for sperm production (spermatogenesis) while LH stimulates the production of testosterone, which is required to continue the spermatogenesis process. Testosterone also plays a role in the development of male characteristics such as muscle mass and strength, fat distribution, bone mass, and sex drive. in defect or imbalance of those hormones lead to male infertility [8]. Owing to the complexity of human spermatogenesis, male infertility is highly complex with extremely heterogeneous phenotype presentations among infertile men. It is currently estimated that known genetic factors such as chromosomal abnormalities, aneuploidies, Y chromosome microdeletions, and single-gene defects are responsible for at least 15–30% of male infertility [9]. Several studies have identified additional genetic factors that include single-gene and multiple-gene defects that are associated with male infertility in the past two decades. However, the etiology remains obscure in a majority of infertile men (~40%), and identification of novel genetic factors linked with idiopathic male infertility is a major research concern [10].

Several studies indicated that gene polymorphisms in different genes can cause male infertility including FSHR, NF-kB1, protamine and ensogene [11–14], as well USP9Y gene.

USP9Y (initially known as Drosophila fat facets related Y) is a single copy gene, located in the AZFa region. USP9Y enzyme belongs to the peptidase C19 family and has role in sperm cell development in mammals. USP9Y enzyme is similar to the ubiquitin-specific proteases, which cleave the ubiquitin moiety from ubiquitin-fused precursors and ubiquitinated proteins [15].

■ MATERIALS AND METHODS

Ethical approval

The thesis project has been approved according to the decision of the Research Committee in the Thi-Qar Health Directorate and facilitation task issued by the Thi-Qar Health Directorate, Department of Training and Development (issue No. 86 on 2025), which are attached to the document of University of Baghdad / Genetic Engineering and Biotechnology Institute (issue No.7380 on 18/2/2025), where the patient's consent is taken verbally when reviewing hospitals designated as infertility unite.

Sample collection

A total of 150 blood and semen samples were collected for this investigation; venous blood samples were taken from infertile patients so as control group samples drawn as follows. About five ml were collected from each sample and placed in a gel and EDTA tubes and left at room temperature for about 30 minutes. The gel tubes containing samples were centrifuge at 4000 RPM for 5 minutes and the serum sample was deposited into Eppendorf tubes, contains approximately 1000 µl and stored in a deep freeze at –20 C°. The serum samples were used for assessment of hormone levels by Enzyme-Linked-Immuno-Sorbent-Assay Technique (ELISA).

Inclusion and exclusion criteria

Infertile patients with secondary infertility, obstructive azoospermia, and varicocele (as determined by clinical examination and ultrasonic waves), as well males with chronic diseases such as cardiovascular disease, diabetes mellitus, and hypertension. and family history.

Statistical analysis

The data of the current study was statistically analysis by using SPSS (Statistical Package of Social Science version 26), based in using one-way ANOVA and LSD, Chi-square, and Odds ratio at p. value <0.05 [16].

■ RESULTS

Levels of hormones in patients and control

Table 1 showed the comparison between different groups in hormonal levels significantly evaluated in patients than control group at p. value <0.01.

Table 1
Comparison between different groups in hormonal levels

SFA	Azoospermia	Oligospermia	Control	p. value	LSD
	Mean±SE				
LH	6.41±0.52[a]	5.45±0.38[a]	3.49±0.17[b]	<0.01**	0.99
FSH	7.94±0.82	7.48±0.74	7.05±0.42	0.663	NS
Testosterone	4.40±0.41[b]	3.57±0.33[b]	6.17±0.25[a]	<0.01**	0.86
Prolactin	16.8±0.94[a]	18.0±0.82[a]	9.45±0.59[b]	<0.01**	2.03
Estradiol	21.7±1.13[a]	16.2±1.42[b]	14.8±1.19[b]	<0.01**	3.20
AMH	3.38±0.36	3.35±0.35	4.20±0.24	0.118	NS

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Genotype distribution and allele frequency at USP9Y gene (rs112694338) SNP in patients and control group

Tables 2 and 3 showed the allele frequency at USP9Y gene (rs112694338) and their relationship with male infertility in both Azoospermia and oligospermia.

Table 2
Genotype distribution and allele frequency at USP9Y gene (rs112694338) SNP in Azoospermia patients and control group

rs112694338 Genotype	Azoospermia, No. 50		Control, No. 50		CalX²	OR 95% CI	p. value
	No.	%	No.	%			
GG wild	11	22.00	25	50.00	8.50	0.28 (0.11–0.67)	<0.01**
GT hetero	30	60.00	20	40.00	4.00	2.25 (1.01–5.00)	0.046*
TT mutant	9	18.00	5	10.00	1.32	1.97 (0.61–6.38)	0.249
Allele Frequency	**Azoospermia**		**Control**		**CalX²**	**OR 95% CI**	
G	41 (0.52)	51.25	45 (0.70)	64.29	2.59	1.97 (0.58–1.12)	0.107
T	39 (0.48)	48.75	25 (0.30)	35.71			

Table 3
Genotype distribution and allele frequency at USP9Y gene (rs112694338) SNP in Oligospermia patients and control group

rs112694338 Genotype	Oligospermia, No. 50		Control, No. 50		CalX²	OR 95% CI	p. value
	No.	%	No.	%			
GG	14	28.00	25	50.00	5.08	0.38 (0.17–0.89)	0.024*
GT	28	56.00	20	40.00	2.56	1.90 (0.86–4.22)	0.109
TT	8	16.00	5	10.00	0.79	1.71 (0.52–5.65)	0.372
Allele Frequency	**Oligospermia**		**Control**		**CalX²**	**OR 95% CI**	
G	42 (0.56)	53.85	45 (0.70)	64.29	1.65	0.64 (0.33–1.25)	0.198
T	36 (0.44)	46.15	25 (0.30)	35.71			

Genotype distribution and allele frequency at USP9Y gene (rs3212291) SNP in patients and control group

Table 4 and 5 showed the allele frequency at USP9Y gene (rs3212291) and their relationship with male infertility in both Azoospermia and oligospermia.

Table 4
Genotype distribution and allele frequency at USP9Y gene (rs3212291) SNP in Azoospermia patients and control group

rs3212291 Genotype	Azoospermia No. 50		Control No. 50		CalX²	OR 95% CI	p. value
	No.	%	No.	%			
CC	12	24.00	33	66.00	17.8	0.16 (0.08–0.39)	<0.01**
GC	29	58.00	15	30.00	7.95	3.22 (1.41–7.35)	<0.01**
GG	9	18.00	2	4.00	5.00	5.25 (1.07–25.7)	0.025*
Allele Frequency	**Azoospermia**		**Control**		**CalX²**	**OR 95% CI**	
C	41 (0.53)	51.90	48 (0.81)	73.85	7.27	0.38 (0.18–0.77)	<0.01**
G	38 (0.47)	48.10	17 (0.19)	26.15			

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Table 5
Genotype distribution and allele frequency at USP9Y gene (rs3212291) SNP in Oligospermia patients and control group

rs3212291 Genotype	Oligospermia No. 50		Control No. 50		CalX²	OR 95% CI	p. value
	No.	%	No.	%			
CC	10	20.00	33	66.00	21.5	0.12 (0.05–0.31)	<0.01**
GC	33	66.00	15	30.00	12.9	4.52 (1.95–10.5)	<0.01**
GG	7	14.00	2	4.00	3.05	3.90 (0.77–19.8)	0.081
Allele Frequency	**Oligospermia**		**Control**		**CalX²**	**OR 95% CI**	
C	43 (0.53)	51.81	48 (0.81)	73.85	7.47	0.38 (0.18–0.76)	<0.01**
G	40 (0.47)	48.19	17 (0.19)	26.15			

■ DISCUSSION

The mean level of LH hormone in Azoospermia was more than in Oligospermia and higher than that of the control mean. Given that the body tries to promote Leydig cell activity by producing more LH, this points to primary testicular failure. The mean level of FSH hormone in Azoospermia and Oligospermia not significant compared to the control group mean.

The mean level of Testosterone hormone in Azoospermia and Oligospermia significantly lower than that of the control mean. considerably lower in both patient groups when compared to controls, suggesting decreased androgenic activity and compromised Leydig cell function, both of which may have an impact on spermatogenesis. The mean level of prolactin hormone in Azoospermia and Oligospermia significantly higher than that of the control mean. considerably higher in the patient's groups. Fertility can be negatively impacted by hyperprolactinemia because it can inhibit gonadotropin-releasing hormone (GnRH), which in turn lowers LH and FSH.

The mean level of Estradiol hormone level in Azoospermia and Oligospermia significantly higher than that of the control mean. modestly elevated in oligospermic and much greater in azoospermia patients when compared to controls. Fertility problems may be exacerbated by endocrine dysregulation or enhanced aromatization of androgens. The mean level of AMH (Anti-Mullerian Hormone) level in Azoospermia and Oligospermia not significant compared to the control mean. There was no discernible difference between the groups, although a downward trend in patients may point to mild Sertoli cell malfunction that needs more research.

The result of this study shows a significant increase in the level of LH in the patient groups which agrees with studies of Nayyfe et al. [17]; Nada and Al-Ahmed [18]; Naznin et al. [19]; Al-Kalabi et al. [20] while this result disagrees with the studies of Alsalman et al. [21]: Sadoon and Al-baidani [22] that suggests there is decrease in the serum hormone levels in LH hormone in infertile male.

Infertile men had significantly higher levels of both FSH and LH than fertile subjects ($P<0.001$), according to a study by Andrabi et al. [23], indicating their roles in fertility.

The FSH level results indicate no significant difference, which is consistent with the study by Khalil and Kadhim [24], but contradicts the findings of Nayyfe et al. [17], who proposed that the FSH level increased, and Yenzeel [25]; Mohammed et al. [26], who found that the FSH level decreased in infertile males compared to fertile males.

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The result of this study shows a significant decrease in the level of Testosterone in both Azoospermia and Oligospermia groups the current study results agree with the results of [27–30] while these results were in disagreed with study of Nistal et al. [31] who found normal levels of Testosterone in infertile men in comparison with fertile men. On the other hand, the study of Palani [32], found no significant difference of Testosterone in infertile men as compared to fertile men. Steroid hormones, such as testosterone, are necessary for the development and maintenance of secondary sexual characteristics as well as initiation and maintenance of spermatogenesis. Taking Testosterone supplements or Testosterone replacement therapy (TRT) for abnormally low Testosterone levels could make testosterone levels spike [33].

There is significant difference (increase) in level of E2 in patient groups which agrees with the study of Sadoon and Al-baidani [22]; Khalil and Kadhim [24]. Another study suggested that the high level of E2 may occur due to some medications and substances like (Antibiotics-herbs or other natural substances, such as gingko or ginseng – phenothiazine which is a medication used for mental health conditions) High estrogen is also could passed down through genes. Other health conditions can raise estrogen levels, such as: stress-weight gain or obesity-tumors-diseases that affect the liver-conditions that affect hormone balances, such as hypogonadism [34].

This study showed a significant increase in serum PRL (prolactin) levels of both Azoospermia and Oligospermia infertile men compared with control group. The results agree with studies of Abbas [29]; Green and Amadi [35]; Kadhim and Abdul-hassan [36] results. The PRL-spermatogenesis connection seems to be mild, although low-normal PRL levels are associated with the best spermatogenetic profile. PRL serum levels could mirror the immunoregulatory status within the testis, suggesting that there is a sort of 'PRL optimal window' reflecting an efficient spermatogenesis. Alternatively, men with good semen parameters might have a higher central dopaminergic tone resulting in low PRL levels [37]. Because of estradiol and prolactin is not routinely measured in men in clinical practice. Advancements in methods to precisely measure estradiol in men and reduce their costs should provide better evidence on this issue and inform clinical practice. New basic and clinical research is required to improve our knowledge of the role of estrogen in male reproductive function and men's health in general.

The hyperprolactinemia could also be the results of elevation in the prolactin levels through increased sensitivity to prolactin-releasing factor and prolactin inhibitory factor and from altered regulation of serotonergic and oradregneric neurons which in turn modulate dopamine release. Adding to that, seizures induce discharges in the neuron cell which stimulate the hypothalamus and increased prolactin secretion by the pituitary gland. The result of the level of AMH hormone shows non-significantly decreased in both Azoospermia and Oligospermia groups compared to control which agrees with the study of Nayyfe et al. [17]; Tunç et al. [38] who suggested that there was a non-significant decrease in the AMH level in infertile male meanwhile which indicates the mild role of AMH hormone when it comes to spermatogenesis process in adult male.

The results of genotypes and alleles frequencies at USP9Y gene (rs112694338) SNP. in controls versus Azoospermia Patients with primary infertility are presented in table 2. The percentage of wild-type GG genotype was significantly ($p \leq 0.01$) lower in-patient group than in controls group (22.0% versus 50.0%, respectively). significantly more common in the azoospermia group (22%), compared to the control group (50%). High statistical

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significance ($p<0.01$) suggests that this genotype protects against azoospermia. Then TT genotype (mutant) was non-significant in the comparison between Azoospermia and control group, not statistically significant, although higher in patients than controls ($p=0.249$). However, this could be may represent a protective factor against the incidence of patients with infertility.

Whereas, the percentage of heterozygous GT genotype was significantly ($p\leq0.046$) higher in patients with Azoospermia than in controls (60.0% versus 40%, respectively). Then genotype may represent a risk factor for the incidence of Iraqi patients with Azoospermia. The control group has a higher frequency of the G allele, which lends greater credence to its potential protective relationship.

Patients are more likely to carry the T allele; however, this difference is not statistically significant ($p=0.107$). The GG and GT genotypes of the USP9Y gene (rs112694338) are statistically significantly associated with azoospermia. While GT may make a person more susceptible, the GG genotype seems to lower the risk. The effect of the T allele is intriguing but not definitive. The percentage of wild-type GG genotype was significantly higher in control group ($p\leq0.024$), 50% of controls had this condition, compared to 22% of azoospermia patients lower in patients' group. A strong protective connection with fertility is shown by a p-value of less than 0.01.

Then TT genotype (mutant) was non-significant in the comparison between the Oligospermic patients and control group, no firm conclusion can be made because the number of patients was higher but not statistically significant ($p=0.249$). therefore, this could be representing a protective factor against the incidence of patients with infertility. However, the comparison percentage of heterozygous GT genotype was also non-significant even though the number of oligospermia was slightly higher than in controls (56.0% versus 40%, respectively). Then genotype G may represent a risk factor for the incidence of Iraqi patients with oligospermia. The results of the three tables above show the frequency of the G and T allele in the three groups the frequencies reveled that G allele is more frequent in control group while the T allele was more frequent in the patient's groups compared the control group this revels that the mutant allele T which is more distributed in Azoospermia mostly and Oligospermia as well consider to be the risk allele and male with this allele are more susceptible to male infertility.

While the G allele is considering a protective allele and male with the G allele are less susceptible to male infertility. Male infertility, namely azoospermia, seems to be linked to the USP9Y gene's rs112694338 polymorphism. While GT and TT genotypes may be associated with an increased risk of infertility, the GG genotype may offer some protection. Although it might not be specific for differentiating between different forms of infertility, these results show the potential importance of USP9Y as a candidate gene in genetic screening for male infertility.

The results above in this study agrees with the study of Nailwal and Chauhan [39], that studied the Analysis of consequences of non-synonymous SNPs of USP9Y gene in human using bioinformatics tools whom they found Y-chromosome is the ubiquitin-specific Protease 9, Y chromosome (USP9Y). In contrast to the current study findings, which point to a possible relationship, the study of Kalhor et al. [40], showed no significant association between rs112694338 and infertility. This disparity might draw attention to effects unique to a population or interactions with additional environmental or genetic factors. The present study findings might suggest that rs112694338 is more prevalent

in the Iraqi population, either as a result of regional founder effects or different genetic architecture.

The genotype and allele frequency distributions for the rs3212291 SNP in the USP9Y gene between azoospermia patients and control (fertile) individuals are displayed in table 4 along with statistical comparisons using p-values, odds ratios (OR), and chi-square (CalX^2). With an odds ratio (OR) of 5.25, the GG genotype has a more than fivefold higher incidence of azoospermia than the CC genotype, and all three genotypes exhibit statistically significant differences between the two groups ($p<0.05$). The percentage of wild-type CC genotype was significantly ($p\leq0.01$) lower in-patient group than in controls group (24.0% versus 66.0%, respectively) which is represent a protective association factor against the incidence of infertility in male. The GG genotype (mutant) was also significant ($p\leq0.025$) in the comparison between Azoospermia and control group (18.0% versus 4.0%, respectively), however this could be representing a risk association factor against the incidence of infertility in male.

Whereas, the percentage of heterozygous mutant GC genotype was significantly ($p\leq0.01$) higher in patients with Azoospermia than in controls (58.0% versus 30.0%, respectively). Then genotype may represent a risk factor for the incidence of Iraqi patients with Azoospermia.

The comparison of allele frequencies also reveals a highly significant difference ($p<0.01$), with the C allele's protective role reinforced by an OR of 0.38 and the G allele raising the likelihood of azoospermia.

The findings show a strong correlation between the risk of oligospermia and the USP9Y gene's rs3212291 polymorphism. The control group had a considerably higher frequency of the wild-type genotype CC (66%) compared to the oligospermic group (20%), indicating that this genotype may be protective against male infertility. On the other hand, oligospermic patients were more likely to have the GC heterozygous genotype (66%) than the control group (30%), suggesting a clear correlation with higher risk (OR=4.52, $p<0.01$). Although oligospermic patients had a higher prevalence of the GG mutant genotype (14%) compared to controls (4%), this difference was not statistically significant ($p=0.081$). In terms of allele frequencies, oligospermic patients had a higher frequency of the G allele (mutant) (48.19%) compared to controls (26.15%), which may indicate a risk allele. Conversely, the wild-type C allele was substantially more prevalent in the control group (73.85%) than in oligospermic patients (51.81%), supporting the idea that the C allele might be protective. All things considered; these results lend credence to the idea that the rs3212291 polymorphism plays a role in the genetic susceptibility to oligospermia.

The findings imply that neither oligospermia nor azoospermia in the group under study are significantly correlated with the rs3212291 SNP in the USP9Y gene. There is no discernible genetic variation associated with either illness, and the genotypes and allele frequencies seem to be comparable between the two patient groups. The results above in this study agrees with the study of Nailwal and Chauhan [39], the results of this study is defiantly with an important use along with the previous studies such as Kalhor et al. [40], that investigate the assessment of five polymorphisms in the USP9Y gene in infertile patients with non-obstructive Azoospermia trying to revel the factors involved in spermatogenesis, including hormones and genes, in order to present a better understanding to the mechanism of NOA in men. Moreover, the study of Cannarella et al.

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[41], that investigate the analysis of 29 targeted genes for non-obstructive Azoospermia USP9Y gene was among those 29 genes also the relation to the VUSs of the USP9Y gene which were the most frequently as they were found in 14 out of 48 patients (29%). In particular, the VUS USP9Y c.7434+14 del was found in 11 patients. They showed varied histological pictures, including Sertoli cell-only syndrome, mixed atrophy, and hypo spermatogenesis, regardless of cryptorchidism or varicocele.

Another study was the assessment of genotype frequencies of thirteen polymorphisms in Y chromosome including two polymorphisms in USP9Y gene which was done by Kim et al. [42] on a group of Korean males. However, the contradiction between old studies of USP9Y gene such as the study of Luddi et al. [43], suggested that Deletions in the azoospermia factor region AZFa on the human Y chromosome and, more specifically, in the region of USP9Y gene have been implicated in infertility associated with oligospermia and azoospermia. This study was capable of characterizing in detail a deletion in AZFa that results in an absence of USP9Y in a normospermia man and his brother and father. The association of this large deletion with normal fertility shows that USP9Y, hitherto considered a candidate gene for infertility and azoospermia, does not have a key role in male reproduction. These results suggest that it may not be necessary to consider USP9Y when screening the Y chromosome of infertile or sub fertile men for microdeletions. Which is the opposite of the study of Krausz et al. [44], that concluded the USP9Y gene has been considered as one of the major Y-linked spermatogenesis genes, based on both its position within the AZFa region and previous reports that correlated USP9Y mutation to severe spermatogenic failure and infertility [45]. This view is now substantially changed because our findings clearly demonstrate that during human spermatogenesis, USP9Y is more likely a fine tuner that improves efficiency, rather than a provider of an essential function. More importantly, the observed natural conceptions suggest that the protein is not required for the final sperm maturation process or for the acquisition of sperm fertilizing ability, providing a new perspective on the role played by the USP9Y gene in male fertility. The importance of the USP9Y gene is still ambiguous, not definitive and inconclusive because of these contradictions about the USP9Y gene therefore lots of studies such as this one is still yet to be made.

■ CONCLUSION

A disturbance in testicular endocrine function is suggested by the hormonal profile of infertile men, especially those who have azoospermia Variants in the USP9Y gene indicate a genetic role in male infertility. The GG genotype of rs112694338 and CC genotype of rs3212291 appear protective, while other genotypes and alleles increase infertility risk.

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Immunological Role of Interleukin-6 and Some of Blood Parameters in Miscarriage Cases

Conflict of interest: nothing to declare.
Authors' contribution: Fatima Sadiq Azeez – conceptualization, data curation, investigation, methodology, project administration, resources, software, visualization, writing – original draft and writing – review & editing; Ali Naeem Salman – conceptualization, supervision, validation, visualization, writing – original draft and writing – review & editing.
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Abstract

Introduction. Miscarriage is a spontaneous loss of pregnancy during the first trimester. It is a common complication with multifactorial etiology.
Purpose. The aim of this study was to investigate the effect of Interleukin-6 (IL-6) on miscarriage.
Materials and methods. The research was conducted at Bent Al-Huda Hospital in Thi-Qar Provence between 1/10/2024 to 1/2/2025. The immunological status of women who experienced miscarriage was evaluated by measuring serum IL-6 levels using the enzyme-linked immunosorbent assay (ELISA) technique. A total of 100 blood samples were analyzed, comprising 50 samples from women who had miscarriages and 50 from healthy controls.
Results. A significant increase ($p \leq 0.05$) in serum IL-6 concentrations in the miscarriage group (12.36±26.42) compared to the control group (9.68±10.03) with a statistically significant difference ($p<0.001$). In addition, complete blood count (CBC) was performed to assess white blood cell (WBC) count and lymphocyte (LYM) levels. A significant elevation in both parameters among women who experienced miscarriage. The WBC count in the miscarriage group was (3.51±11.27) compared to (2.10±9.56) in the control group ($p=0.004$), while LYM levels were (0.91±2.44) in the miscarriage group versus (0.54±1.96) in the control group ($p=0.002$).
Conclusion. Data indicates that elevated levels of cytokine IL-6 play a fundamental and influential role in cases of miscarriage.
Keywords: miscarriage, IL-6, lymphocytes, immune cells, pregnancy

■ INTRODUCTION

Miscarriage, defined as the spontaneous loss of pregnancy during the first trimester, remains a common complication with multifactorial causes [1]. It is classified into various types, including threatened, incomplete, complete, recurrent, and missed abortion [2]. Although factors such as chromosomal abnormalities, hormonal imbalances, infections, and lifestyle contribute to miscarriage, the exact mechanisms often remain unclear [3, 4].

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Cytokines are critical regulators of immune communication and response during pregnancy. Interleukin-6 (IL-6), a multifunctional cytokine, plays a key role in immune modulation and inflammatory processes by activating B cells and other immune pathways via the gp130 receptor complex [5, 6].

Immune cells, such as white blood cells (WBCs) and lymphocytes (LYM), play a central role in regulating the body's inflammatory response. Upon exposure to pathogens or cellular injury [7], these cells initiate a cascade of immune reactions aimed at protecting tissues and restoring homeostasis. However, excessive immune activation or imbalance between innate and adaptive responses may lead to tissue damage and immune-related disorders. In the context of pregnancy, such Dysregulation in immune function has been associated with an increased risk of miscarriage, highlighting the critical importance of tightly controlled immune responses during gestation [8].

■ MATERIALS AND METHODS

Design of Study

A total of 50 blood samples were collected from women who experienced miscarriage at Bint Al-Huda Hospital in Thi-Qar Governorate. Additionally, 50 samples were obtained from healthy individuals to serve as the control group. Sample collection and data acquisition were conducted from 1/10/2024 to 1/2/2025.

Collection of Blood Samples

Blood samples 5 ml were placed in gel tubes and allowed to clot at 37 °C for 10–25 minutes, then centrifuged at 3000 rpm for 15–20 minutes to obtain serum [9]. This method was used in the current study to measure IL-6 levels. The Enzyme-Linked Immunosorbent Assay (ELISA) technique was used to measure the levels of IL-6 in the serum of all samples. The ELISA kit is manufactured by (Fine test – China). In addition, a portion of the collected blood was transferred into an EDTA tube to be used for measuring white blood cell (WBC) and lymphocyte (LYM) counts.

Principle of assay

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti-IL-6 antibody was pre-coated onto the 96-well plate. The biotin conjugated anti IL-6 antibody was used as the detection antibody. The standards and pilot samples were added to the wells subsequently. After incubation, unbound conjugates were removed by wash buffer. Then, biotinylated detection antibody was added to bind with IL-6 conjugated on coated antibody. After washing off unbound conjugates, HRP-Streptavidin was added. After a third washing, TMB substrates were added to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that turned yellow after adding a stop solution. Read the O.D. absorbance at 450nm in a microplate reader. The concentration of IL-6 in the sample was calculated by drawing a standard curve. The concentration of the target substance is proportional to the OD450 value.

In addition, white blood cell (WBC) and lymphocyte (LYM) counts were also assessed to evaluate the immune status of the participants. These hematological parameters were measured using a fully automated hematology analyzer, based on impedance or flow cytometry methods.

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Statistical analysis

The data underwent statistical analysis using SPSS, version 23.0. Data are represented as the mean±SD. Comparison of a group of differences in numerical variables was estimated by t-test Where the level of significance was measured by the P value at a significant level (P.value ≤0.05) [10].

■ RESULTS

Interleukin-6

IL-6 in women who experienced miscarriage was elevated, reaching 12.36±26.42 compared to the control group, which had an IL-6 level of 9.68±10.03 (p<0.001), indicating a strong association between elevated IL-6 levels and miscarriage cases (Table 1).

Table 1
Comparison of the levels of IL-6 among two studied T. value groups

Parameter	Patients, n=50	Control, n=50	t-test	p-value
IL-6	12.36±26.42	9.68±10.03	5.26	<0.001

WBC and Lym levels

The results indicated that WBC count and lymphocyte levels were significantly elevated in women who experienced miscarriage, with WBC at 3.51±11.27 and LYM at 0.91±2.44, compared to the control group where WBC was 2.10±9.56 and LYM was 0.54±1.96. Statistically significant differences were observed for WBC and LYM, with p-values of 0.004, 0.002 respectively, suggesting a potential association between increased inflammatory response and the occurrence of miscarriage (Table 2).

Table 2
Comparison of WBC and LYM Counts between Abortion Cases and Control Group

Parameter	Patients, n=50	Control, n=50	t-test	p-value
WBC	3.51±11.27	2.10±9.56	2.95	0.004
LYM	0.91±2.44	0.54±1.96	3.20	0.002

■ DISCUSSION

IL-6 is a multifunctional cytokine produced by various cell types, including T cells, B cells, lymphocytes, monocytes, and others [11]. It exerts its biological effects through the interleukin-6 receptor (IL-6R) expressed on the cell surface. Upon binding to IL-6, this receptor associates with the signal-transducing component glycoprotein 130 (gp130, ~130 kDa), leading to dimerization and subsequent activation of intracellular signaling pathways.Physiologically, IL-6 is involved in the regulation of hematopoiesis and coordination of both innate and adaptive immune responses. Additionally, IL-6 plays a critical role in metabolic regulation, neuronal development and survival, as well as in the progression and maintenance of various types of cancer. Although IL-6 is predominantly recognized as a pro-inflammatory cytokine, several studies have demonstrated its protective functions under certain conditions [12]. Moreover, IL-6 has significant roles in the immune system and inflammatory responses. It is also implicated in embryo implantation, fetal development, and placental regulation [13, 14].

Dysregulation of IL-6 expression or signaling may lead to numerous pregnancy-related complications [15]. Increasing evidence suggests that IL-6 plays a dual role in pregnancy,

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contributing to the balance between fetal tolerance and protective immune responses. However, disruption of this balance may lead to serious complications such as miscarriage.

Multiple studies have reported a significant elevation in IL-6 levels among women who experienced miscarriage, particularly during the second trimester, compared to normal pregnancies. It is believed that excessive IL-6 expression leads to overactivation of the maternal immune system, thereby disturbing the finely regulated immunological environment essential for embryo implantation and pregnancy maintenance [16].

The current study aligns with the findings of AL-Dulymi et al. [17], who reported increased IL-6 concentrations and notable histopathological alterations in the placenta of women who experienced first-trimester miscarriage. These findings further reinforce the hypothesis that dysregulation of IL-6 may contribute to immune imbalance and pregnancy failure.

The study indicates a significant increase in the number of white blood cells and lymphocytes in women who have experienced miscarriage compared to the control group. The research conducted by Al-Husban et al. [18] and Al-Ubaydi et al. [13]. Alterations in inflammatory blood markers, such as elevated white blood cell (WBC) count, may be associated with an increased risk of miscarriage, particularly during the first and second trimesters of pregnancy [19].

■ CONCLUSION

Data indicates that elevated levels of the cytokine IL-6 play a fundamental and influential role in the pathogenesis of miscarriage. IL-6, as a key pro-inflammatory mediator, can disrupt the maternal immune tolerance necessary for successful pregnancy by promoting an imbalance in T-cell subsets (favoring Th17 over Treg), enhancing local inflammation, and impairing trophoblast function.

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Uterine Artery Doppler Screening in the Second Trimester for High-risk Pregnancies as a Predictor of Adverse Perinatal Outcomes

Conflict of interest: nothing to declare.
Authors' contribution: Afrah Najem Jassem – conceptualization, data curation, investigation, methodology, project administration, resources, software, validation, visualization, writing – original draft and writing – review & editing; Riyadh Adel Jaed Abdulazeez – conceptualization, supervision, validation, visualization, writing – original draft and writing – review & editing; Alaa Hussein Ali AL-Naser – conceptualization, data curation, supervision, validation, visualization, writing – original draft and writing – review & editing.
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Abstract

Introduction. Over the past 20 years, Doppler ultrasonography monitoring of the uterine artery, a normal non-invasive procedure, has been suggested as a predictive diagnostic for unfavorable pregnancy outcomes in high-risk pregnancies throughout the second and third trimesters of pregnancy.
Purpose. To assess the role of using uterine artery Doppler screening in the 2nd trimester for high-risk pregnancies in predicting adverse perinatal outcomes.
Materials and methods. A prospective observational study conducted at Bint Al-Huda Teaching Hospital during the period of one year from 1st of March 2024 till 1st of March 2025. It included 118 pregnant females diagnosed as high-risk pregnancy and underwent uterine artery Doppler screening by a radiologist. All pregnant women were followed by gynecologist till delivery, then information obtained from the gynecologist about pregnancy outcome.
Results. In this study, 26.3% of pregnant women developed adverse outcome. The most common cause of being a high-risk group was anemia (49.2%), followed by young age (<19 years) (28.8%). Bilateral notches were seen significantly higher in women with adverse pregnancy outcome. Means of PI and RI were significantly higher in women with adverse pregnancy outcome.
Conclusion. Problems with adverse pregnancy outcomes were prevalent in high-risk pregnancies. Doppler ultrasound measurements of bilateral notches, PI, and RI are useful predictors of these issues.
Keywords: high-risk, doppler, pregnancy, ultrasound, uterine

■ INTRODUCTION

Any pregnancy that increases the mother's or fetus's risk of experiencing health issues throughout pregnancy and delivery is considered high-risk [1]. According to estimates,

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there were 211 maternal fatalities worldwide in 2017 as a result of pregnancy or delivery problems for every 100,000 live births [2]. An estimated 1.3 million maternal deaths among women occurred in the past 20 years, making up 12% of all maternal deaths worldwide [3]. Identifying at-risk pregnancies has been the main goal of maternal and childcare programs in order to shield mothers from obstetric difficulties during childbirth [4]. Risk assessment is a key component of antenatal care (ANC) and has demonstrated benefits in improving maternal and perinatal outcomes [5]. Because preeclampsia and fetal growth retardation/small for gestational age are complicated disorders with multiple subtypes, underlying causes, and risks for unfavorable outcomes, it can be difficult to predict their consequences [6]. A vital diagnostic tool for identifying and treating a variety of illness conditions is Doppler ultrasonography. As technology has advanced and gotten more affordable, Doppler ultrasonography has become more and more popular. Furthermore, the use of point-of-care ultrasonography as a diagnostic technique has grown [7, 8]. Doppler ultrasonography study of the uterine artery, a non-invasive standard procedure, has been suggested as a predictor diagnostic for unfavorable pregnancy outcomes in high-risk pregnancies for the past 20 years. Because placental function and uterine artery Doppler are strongly associated, placental malfunction may be identified early [9]. For women who are at risk of getting pregnant, Doppler ultrasound is a useful tool for controlling their condition, predicting pregnancy, and helping with delivery decisions [10]. Gestational age and Doppler indices were recorded in a late pregnancy. When two arteries are examined, more information is revealed than when only one is examined [11]. A notch (= postsystolic incision) after 24+0 weeks of gestation and/or a high pulsatility index (PI) >95th percentile is generally regarded as problematic findings in the uterine artery during the 2nd trimester Doppler ultrasound [12]. The aim of this study is to assess the role of using uterine artery Doppler screening in the 2nd trimester for high-risk pregnancies in predicting adverse perinatal outcomes.

■ MATERIALS AND METHODS

Study design and setting

This was a prospective observational study conducted at Radiology Department of Bint Al-Huda Teaching Hospital in Thi-Qar Province during the period of one year from 1st of March 2024 till 1st of March 2025.

Study population and sample size

This study included 118 pregnant females diagnosed as high-risk pregnancy at Gynecology and Obstetrics Department and referred at 2nd trimester of pregnancy to the radiology department for uterine artery Doppler screening by a radiologist. High-risk pregnancy includes one or more conditions as hypertensive diseases, anemia, intrauterine growth restriction, maternal age ≤19 years or ≥35 years, gestational DM, Rhesus (Rh) incompatibility, multiple gestation, and history of still birth. Pregnant women who refused to participate in this study or loss to follow up were excluded. A questionnaire was applied to all enrolled patients to collect the needed information. It includes questions to gather information about the demographic characteristics of examined women, obstetrical and gynecological history, surgical history, and Doppler ultrasound examination data.

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Workup

Uterine artery Doppler screening was performed for all included women during the 2nd trimester of pregnancy. Uterine arteries flow velocity waveforms were obtained using voluson E6, GE company, USA) ultrasound machine, 2–5 MHz transducer. The high-pass filter was set at 100 Hz. Before the uterine artery was divided into branches, flow velocity waveforms were taken from the uterine artery close to the external iliac artery. Each artery's three to five successive waveforms were captured, the pictures were frozen, and the PI and S/D ratio were computed. Additionally, noted was the existence or lack of early diastolic notches. All ultrasonography and Doppler studies were carried out by a radiologist.

Outcome

All pregnant women were followed by gynecologist till delivery, then information obtained from the gynecologist about pregnancy outcome. The women then divided into two groups as follow:

- Abnormal group: included women with pregnancy outcome including preeclampsia, eclampsia, HELLP, or SGA;
- Normal group: included women with normal pregnancy outcome.

Ethical considerations and official approvals

The study was conducted in accordance with the ethical standards of the Scientific Committee of College of Medicine / Thi-Qar University.

Statistical analysis

Version 26 of the Statistical Package for Social Sciences (SPSS) was used to analyze the data. The data was displayed as ranges, means, and SD. Percentages and frequencies used to display categorical data. Accordingly, the continuous variables were compared using an independent t-test (two-tailed). The relationship between the preliminary diagnosis and specific facts was evaluated using the Chi square test. For PI and RI as predictors of unfavorable pregnancy outcomes, a receiver operating characteristic (ROC) curve analysis was created. A P-value of less than 0.05 was regarded as significant.

■ RESULTS

In this study, age ranged from 17 to 44 years with a mean of 29.42±6.9 years, 60.2% were living in rural area, 48.3% were finished primary level of education, 71.2% were housewives, 44.9% were overweighed, 66.9% were multipara, and 24.6% had previous history of hypertension. This study showed that 26.3% of pregnant women developed adverse outcome as 15.3% developed preeclampsia, 10.2% delivered SGA baby, 6.8% had preterm labor, and 1.7% diagnosed with HELLP (Table 1).

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Table 1
Distribution of study patients by general characteristics

Variable		No. (n=118)	%
Age (Year)			
<25		37	31.3
25–34		48	40.7
≥35		33	28.0
Residence			
Urban		47	39.8
Rural		71	60.2
Educational Level			
Illiterate		21	17.8
Primary School		57	48.3
Secondary School		18	15.3
Higher Education		22	18.6
Occupation			
Employee		34	28.8
Housewives		84	71.2
BMI Level			
Normal		16	13.6
Overweight		53	44.9
Obese		49	41.5
Parity			
Nullipara		39	33.1
Multipara		79	66.9
Past medical history			
No		74	62.7
Hypertension		29	24.6
Diabetes mellitus		15	12.7
Pregnancy outcome			
Normal		87	73.7
Adverse outcome		31	26.3
Adverse outcome	Preeclampsia	18	15.3
	SGA	12	10.2
	Preterm labor	8	6.8
	HELLP	2	1.7

As shown in figure 1, the most common cause of being a high-risk group was anemia (49.2%), followed by young age (<19 years) (28.8%).

Table 2 shows that bilateral notches were seen in 58.1% of women with adverse pregnancy outcome and in 27.6% of those with normal outcome and this difference was statistically significant (P=0.002). Means of PI and RI were significantly higher in women with adverse pregnancy outcome than that in those with normal pregnancy outcome (1.59 versus 1.21, P=0.001 and 0.81 versus 0.49, P=0.001 respectively).

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Fig. 1. Causes for being a high-risk group

Table 2
Comparison in means of PI and RI according pregnancy outcome

Parameter	Pregnancy outcome		Total (%) n=118	P-Value
	Adverse (%) n=31	Normal (%) n=87		
Bilateral Notches				
Yes	18 (58.1)	24 (27.6)	42 (35.6)	0.002
No	13 (41.9)	63 (72.4)	76 (64.4)	
	Mean±SD	**Mean±SD**		
PI	1.55±0.44	1.07±0.32	0.001	
RI	0.81±0.12	0.52±0.08	0.001	

ROC curve analysis was constructed for PI and RI as predictors for pregnancies adverse outcomes. The cut point of PI was 1.46 and of RI was 0.69. Hence, PI >1.46 and RI >0.69 are predictive for pregnancies adverse outcomes, as a large significant area under the curve (AUC=88.1% and 76.8% respectively) indicating significant association between higher levels of PI and RI and developing pregnancies adverse outcomes. PI was 77.4% sensitive, 58.6% specific, and 63.6% accurate in predicting pregnancies adverse outcomes. RI was 80.6% sensitive, 70.1% specific, and 72.9% accurate in predicting pregnancies adverse outcomes as shown in figures 2 and 3 and table 3.

Table 3
Diagnostic accuracy of PI and RI as predictors for adverse outcomes

Doppler ultrasound parameters	Cut-off value	Sensitivity	Specificity	PPV	NPV	Accuracy
PI	1.46	77.4%	58.6%	40.0%	87.9%	61.6%
RI	0.69	80.6%	70.1%	49.0%	91.0%	72.9%

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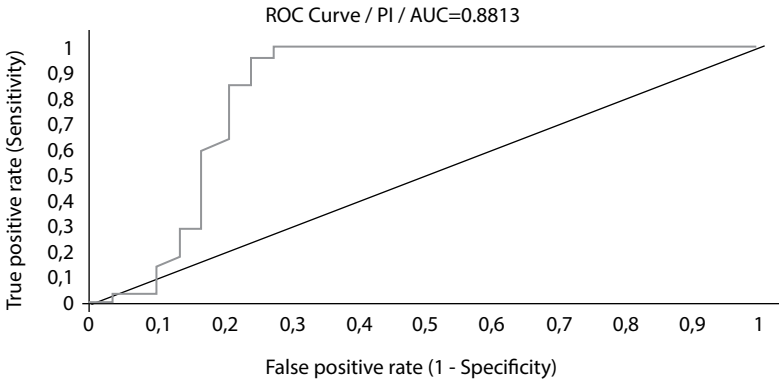

Fig. 2. ROC curve for PI in predicting adverse outcomes

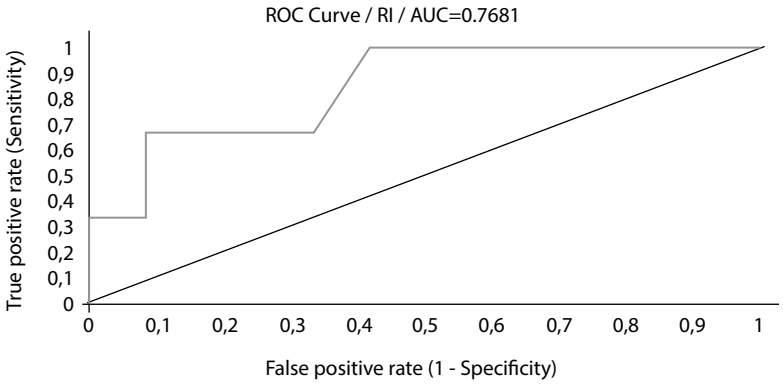

Fig. 3. ROC curve for RI in predicting adverse outcomes

■ DISCUSSION

The uterine artery Doppler is a desirable mid-pregnancy screening test. The test can theoretically distinguish between pregnancies that are at premature delivery and a variety of clinical problems, such as intrauterine growth retardation, abruption, and preeclampsia, that are caused by chronic placental illness [13]. Doppler has made it feasible to investigate fetal circulation non-invasively because aberrant fetal circulation is thought to be a significant contributing element to fetal growth restriction, both as a cause and an indicator [14]. Over the past 20 years, there has been evidence linking uterine artery Doppler indices recorded in the first trimester to an increased risk of adverse late pregnancy outcomes, including preterm birth, SGA, and preeclampsia. Fetal needs eventually outweigh the placenta's ability to support growth and development as pregnancy goes on, and these disorders are most likely the result of the placenta's failure to establish proper circulation in the uterus early in fetal life [15].

In the current study, means of PI and RI were significantly higher in women with adverse pregnancy outcome than normal pregnancy outcome. PI >1.46 and RI >0.69 are predictive for adverse outcomes and bilateral notches were significantly higher in women with adverse pregnancy outcome than those with normal outcome.

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Similar findings noticed in studies conducted by Ratiu D et al in 2019 [16], Kumar KM et al. in 2023 [17], and Lee JY et al. in 2025 [15] when they stated that patients with bilateral high PI or RI showed a significantly higher prevalence rate of adverse pregnancy outcomes and presence of a notch showed a significantly higher prevalence of adverse pregnancy outcomes represented by SGA IUGR at birth, while Shirazi et al. didn't find a significant relationship between RI and these adverse outcomes [18]. Kamur and colleagues in their study showed approaching results as the cutoff point of the uterine artery PI was ≥1.3 and RI was ≥0 in detecting preeclampsia [17]. Adibi A et al study in 2020 showed cutoff point for RI of 0.79 giving comparable results to that published in the current one, while higher cutoff point for PI of 1.99 [19]. Differences in study populations enrolled in each study, gestational ages at assessment, and specific adverse outcomes evaluated can all lead to variations in cut-off values and predicted accuracy between studies.

Given the association between placenta vascular resistance and pregnancy outcomes, Doppler velocimetry has been used to identify women who may experience pregnancy difficulties. Most adverse pregnancy outcomes have been demonstrated to be caused by the placenta, and Doppler velocimetry can detect fetal damage before other antepartum diagnostics [20]. To evaluate the perfusion and vascular sufficiency of the placental bed, changes in the Doppler waveform and Doppler indices – PI, RI, and systolic/diastolic (S/D) ratio – of a few maternal arteries were employed. Even though arterial flow velocity waveforms are best described by these indices, PI provides a more accurate estimation of the waveform's characteristics than RI and S/D ratio. This may be explained by the fact that vascular resistance and PI have a linear relationship, whereas increased vascular resistance and RI and S/D ratio have a parabolic relationship [21].

The main mechanism that encourages the remodeling of the uterine spiral arteries into low-resistance vessels during a typical pregnancy, ensuring an adequate blood supply to the growing fetus and placenta, is the invasion of the maternal spiral arteries by extra-villous trophoblasts [22]. High-risk pregnancies often lack or impair this remodeling process, which causes the uterine arteries to continue to have high-resistance blood flow. The presence of a "notch" in the waveform's early diastolic phase suggests that both uterine arteries are impacted, suggesting a more extensive restriction in placental blood flow. Nonetheless, bilateral uterine artery notching is a powerful sign of compromised placental perfusion and is associated with a number of unfavorable pregnancy outcomes. Doppler ultrasound's early detection enables medical professionals to give appropriate monitoring and interventions, potentially improving outcomes for both the mother and the fetus [23, 24].

■ CONCLUSION

Adverse pregnancy outcomes were prevalent issues in high-risk pregnancies. Doppler ultrasound measurements of bilateral notches, PI, and RI are useful predictors of these issues. In order to keep an eye out for potential complications like preeclampsia, early and routine tests are strongly advised for high-risk pregnant women, especially those with anemia and young maternal age. Clinical practice should regularly employ Doppler ultrasound as a prediction technique to identify pregnancies at risk for adverse outcomes.

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Placental Abruption: Risk Factors and Consequences

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Abstract

Introduction. Placental abruption, affecting 0.4–1% of pregnancies, is linked to smoking, hypertensive disorders, thrombophilia, infection, trauma, and uterine or placental abnormalities. Perinatal consequences include preterm birth, low birthweight, fetal growth restriction, asphyxia, stillbirth, and high mortality, especially with extensive placental separation.
Purpose. This study aims to investigate placental abruption in Basrah, focusing on its prevalence, maternal risk factors, related complications, and adverse perinatal outcomes to guide improved maternal-fetal care.
Materials and methods. This cross-sectional study was conducted on 70 women diagnosed with placental abruption at Basrah Maternity and Children Hospital and Al-Basrah Teaching Hospital over one year, from September 2024 to October 2025. Data covered sociodemographic, obstetric, medical and fetal variables. Diagnosis relied on clinical history and examination, with ethical approvals obtained.
Results. The study of 70 women with placental abruption showed a mean age of 28.6±7.61 years, with most from rural areas, unemployed, and overweight or obese. Nearly half had miscarriages, 30% prior abruption, and 54.3% anemia. Vaginal bleeding (67.1%) and abdominal pain (64.3%) were common presentations. Maternal complications included blood transfusion (48.6%) and intensive care unit admission (27.1%), while cesarean delivery predominated (71.4%). Fetal outcomes showed 42.9% low birthweight, 12.9% stillbirth, and 62.9% neonatal respiratory distress.
Conclusions. Placental abruption in Basrah is associated with late diagnosis, maternal complications, and poor neonatal outcomes. Contributing factors include young maternal age, anemia, obesity, and low socioeconomic status, increasing risks of morbidity and mortality.
Keywords: placental abruption, preterm birth, low birthweight, asphyxia, stillbirth

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■ INTRODUCTION

Placental abruption, classically defined as the complete or partial separation of a normally implanted placenta before delivery, occurs in 0.4–1% of pregnancies. The incidence varies slightly in different populations, and has been increasing in some studies but not all [1]. At least 50 different risk factors or risk markers for placental abruption have been reported, with smoking, preeclampsia and history of previous placental abruption being the strongest. Although many risk factors or risk markers are known, the cause of placental abruption often remains unexplained [2].

Placental abruption is one of the most significant causes of maternal morbidity and perinatal mortality [3].

Both maternal and perinatal risks associated with placental abruption depend on the severity of abruption. Maternal peripartum risks include obstetric hemorrhage, need for blood transfusion, emergency hysterectomy, disseminated intravascular coagulopathy (DIC), renal failure and even maternal death [4].

Fetal risks are associated with intrauterine growth restriction (IUGR), low birth weight, preterm delivery, asphyxia, stillbirth and perinatal death. Fetal survival depends not only on the severity of the abruption but also on the gestational age [5].

Several risk factors have been identified, including maternal smoking, hypertensive disorders (especially preeclampsia), trauma, premature rupture of membranes, and thrombophilias [3, 6]. Hypertensive disorders and smoking are among the strongest and most consistent risk factors, and the risk increases when these coexist [7]. Despite recognition of these risk factors, the precise etiology of placental abruption remains multifactorial and incompletely understood, with genetic, inflammatory, and vascular factors possibly contributing [2, 8].

This study aims to identify the risk factors and maternal as well as fetal outcomes associated with placental abruption among pregnant women admitted to Basrah Maternity and Child Hospital.

■ MATERIALS AND METHODS

This cross-sectional study was conducted on 70 women diagnosed with placental abruption at Basrah Maternity and Children Hospital and Al-Basrah Teaching Hospital over one year, from September 2024 to October 2025.

Participants were evaluated based on the following variables: maternal age, residency, educational level, employment status, monthly household income, weight, height, body mass index (BMI), gravidity, parity, history of previous placental abruption, history of miscarriage, history of labor, history of cesarean section, pre-existing medical conditions, smoking during pregnancy, type of pregnancy, trauma during pregnancy, placental abnormalities, use of assisted reproductive techniques, presenting symptoms, timing of diagnosis, maternal outcomes, hysterectomy requirement, ICU admission, fetal gender, fetal outcomes, neonatal complications, and mode of delivery.

The study population included pregnant women who presented to the labor and delivery units at Basrah Maternity and Children Hospital and Al-Basrah Teaching Hospital during the study period and were diagnosed with placental abruption.

Diagnosis of placental abruption was established based on the patient's clinical history and physical examination.

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Verbal consent was obtained from all participants before inclusion in the study. Additionally, official approval was obtained from the ethical committee at the University of Basrah College of Medicine.

Statistical analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 24. Descriptive statistics, including frequencies and percentages, were used to summarize the data.

■ RESULTS

The study includes 70 pregnant women. The mean age of the study participants was 28.65±7.61 years, ranging between 16 and 43 years. Regarding residency, 47.1% were from urban areas and 52.9% from rural areas. Concerning educational level, 5.7% were illiterate, 38.6% had primary education, 31.4% secondary, and 24.3% higher education. Employment status showed that 31.4% were employed and 68.6% unemployed. Monthly income was reported as low in 30.0%, moderate in 55.7%, and high in 14.3%. The mean weight was 71.85±11.0 kg, the mean height was 160.7±4.1 cm, and the mean BMI was 27.71±3.46. Based on BMI categories, 25.7% were normal, 40.0% overweight, and 34.3% obese. As shown in Table 1.

Table 1
Sociodemographic and anthropometric characteristics of the study participants (n=70)

Variables		No.	%
Age	Mean±SD Range	28.65±7.61 (16–43)	
Residency	Urban	33	47.1
	Rural	37	52.9
Educational level	Illiterate	4	5.7
	Primary	27	38.6
	Secondary	22	31.4
	Higher education	17	24.3
Employment status	Employed	22	31.4
	Unemployed	48	68.6
Monthly income	Low	21	30.0
	Moderate	39	55.7
	High	10	14.3
Weight	Mean±SD	71.85±11.0	
Height	Mean±SD	160.7±4.1	
BMI	Mean±SD	27.71±3.46	
	Normal	18	25.7
	Overweight	28	40.0
	Obese	24	34.3

Table 2 shows the Obstetric and Medical History of the Study Participants. Parity showed that 28.6% were nullipara, 65.7% had 1–4 children, and 5.7% had more than 5. A history of miscarriage was reported in 47.1%, previous placental abruption in 30.0%, preterm labor in 21.4%, and cesarean section in 35.7%. Preexisting medical conditions

included chronic hypertension in 17.1%, preeclampsia in 15.7%, thrombophilia in 5.7%, diabetes mellitus in 14.3%, and anemia in 54.3%, while 25.7% had no medical conditions. Smoking during pregnancy was reported by 2.9%. Regarding the type of pregnancy, 88.6% were singleton and 11.4% multiple. Trauma during pregnancy occurred in 5.7%, placental abnormalities were diagnosed in 7.1%, and assisted reproductive techniques were used in 8.6%.

Table 2
Obstetric and medical history of the study participants (n=70)

Variables		No.	%
Parity	Nullipara	20	28.6
	1–4	46	65.7
	>5	4	5.7
History of miscarriage		33	47.1
Previous placental abruption		21	30.0
History of preterm labour		15	21.4
History of Cesarean Section		25	35.7
Preexisting medical conditions	Chronic hypertension	12	17.1
	Preeclampsia	11	15.7
	Thrombophilia	4	5.7
	Diabetes mellitus	10	14.3
	Anemia	38	54.3
	None	18	25.7
Smoking during pregnancy		2	2.9
Type of Pregnancy	Singleton	62	88.6
	Multiple	8	11.4
Trauma During Pregnancy		4	5.7
Placental Abnormalities Diagnosed		5	7.1
Use of Assisted Reproductive Techniques		6	8.6
Total		70	100.0

Table 3 shows the clinical presentation and timing of diagnosis in cases of placental abruption. The presenting symptoms included vaginal bleeding in 67.1%, abdominal pain in 64.3%, uterine tenderness in 34.3%, and fetal distress in 18.6%. The timing of diagnosis was antenatal through routine scans in 45.7% and during labor in 54.3%.

Table 3
Clinical Presentation and timing of diagnosis in cases of placental abruption (n=70)

Variables		No.	%
Presenting symptoms	Vaginal bleeding	47	67.1
	Abdominal pain	45	64.3
	Uterine tenderness	24	34.3
	Fetal distress	13	18.6
Timing of Diagnosis	Antenatal (routine scan)	32	45.7
	During labor	38	54.3
Total		70	100.0

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Table 4 shows maternal outcomes and mode of delivery among study participants. Maternal outcomes included blood transfusion in 48.6%, hysterectomy in 14.3%, and ICU admission in 27.1%. The mode of delivery was normal vaginal delivery in 28.6% and cesarean section in 71.4%.

Table 4
Maternal outcomes and mode of delivery among study participants (n=70)

Maternal Outcomes		No.	%
Blood transfusion		34	48.6
Hysterectomy		10	14.3
ICU admission		19	27.1
Mode of Delivery	NVD	20	28.6
	C/S	50	71.4

Table 5 shows fetal and neonatal outcomes in cases of placental abruption. Fetal gender was male in 54.3% and female in 45.7%. Birth weight was normal in 57.1% and low (<2.5 kg) in 42.9%. Fetal status showed 87.1% live births and 12.9% stillbirths. Apgar score at 1 minute was normal (≥7) in 34.3% and abnormal (<7) in 65.7%. Neonatal complications included preterm birth in 15.7%, respiratory distress syndrome in 62.9%, hypoxia in 14.3%, and none in 21.4%.

Table 5
Fetal and Neonatal Outcomes in Cases of Placental Abruption (n=70)

Variables		No.	%
Fetal gender	Male	38	54.3
	Female	32	45.7
Birth weight	Normal	40	57.1
	Low (<2.5 kg)	30	42.9
Fetal status	Live birth	61	87.1
	Stillbirth	9	12.9
Apgar score at 1 minute	Normal (≥7)	24	34.3
	Abnormal (<7)	46	65.7
Neonatal Complications	Preterm birth	11	15.7
	Respiratory distress syndrome	44	62.9
	Hypoxia	10	14.3
	None	15	21.4

■ DISCUSSION

Placental abruption remains one of the most serious complications in obstetrics, associated with significant maternal and perinatal morbidity and mortality worldwide [9]. Its unpredictable onset and rapid progression make it a critical concern for clinicians, particularly in low- and middle-income countries where antenatal screening and emergency obstetric services may be limited [10].

The mean age of participants was 28.65 years, which aligns with a report by Oltean et al. indicating that placental abruption frequently affects women in their late twenties to early thirties. Nearly half of the participants (47.1%) resided in urban areas, while a slightly higher proportion (52.9%) came from rural regions [11].

Regarding education, a notable proportion had only primary education (38.6%), while 5.7% were illiterate. This is consistent with studies from developing countries, which show that lower educational attainment is significantly associated with poor pregnancy outcomes, including placental complications [12].

Anthropometric analysis revealed a mean BMI of 27.7, with 40% overweight and 34.3% obese. These figures are noteworthy since maternal obesity has been reported by Salihu et al. (2019) as an independent risk factor for placental abruption [13]. The high prevalence of overweight and obesity in our cohort highlights the role of emerging metabolic and nutritional challenges in Iraq as contributors to obstetric complications.

In this study, 28.6% of women were nulliparous, while 65.7% had 1–4 children. This distribution reflects global trends where placental abruption is common across all parity levels but may be more pronounced in multiparous women, as evidenced by Ananth et al. [14]. Previous placental abruption was present in 30%, which is substantially higher than the recurrence rate of 10–17% reported in Western cohorts, as evidenced by Ruiter et al. [15]. This higher recurrence could be due to differences in antenatal monitoring, early recognition, or management strategies in our setting.

Preexisting medical conditions were common, particularly anemia (54.3%), hypertension (17.1%), and diabetes mellitus (14.3%). Anemia prevalence was strikingly higher compared with Indian reports (32%) [16], reflecting the endemic nature of anemia in Iraqi women of reproductive age.

The most frequent presenting symptoms were vaginal bleeding (67.1%) and abdominal pain (64.3%). These rates are consistent with findings from Ethiopia, where vaginal bleeding occurred in 70% of cases and abdominal pain in 62% [17].

Diagnosis was made antenatally in 45.7% and intrapartum in 54.3%. This relatively high rate of intrapartum diagnosis reflects the limited availability of routine antenatal ultrasound and may contribute to poorer outcomes compared with high-income countries, where most cases are identified before labor onset [18].

The maternal outcomes in this study were concerning, with blood transfusion required in 48.6%, hysterectomy in 14.3%, and ICU admission in 27.1%. These figures are higher than those reported in developed countries, where hysterectomy rates are usually below 5% [19]. This difference likely reflects delays in accessing tertiary care and higher rates of severe abruption in our population. The cesarean delivery rate (71.4%) was also higher than reported averages (50–60%) by Downes et al., possibly due to the critical need for urgent intervention to prevent maternal and fetal compromise [20].

Perinatal outcomes were poor, with stillbirth in 12.9% and low birth weight (<2.5 kg) in 42.9%. These rates are higher compared with global estimates of 5–10% for stillbirth and 20–30% for low birth weight in abruption cases as reported by McClure et al. [21].

Apgar scores were markedly affected, with 65.7% of neonates scoring <7 at one minute, indicating significant intrapartum hypoxia. This is notably worse than the 40–50% abnormal Apgar rates reported in some counties [22, 23].

■ CONCLUSION

Placental abruption in Basrah is associated with late diagnosis, maternal complications, and poor neonatal outcomes. Contributing factors include young maternal age, anemia, obesity, and low socioeconomic status, increasing risks of morbidity and mortality.

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Screening for Iron Deficiency Among Pregnant Women in Basrah Governorate

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Authors' contribution: Nawal Mohsin Hassoon – conceptualization, data curation, investigation, methods, project administration, resources, software, writing – original draft and writing – review & editing; Ghufran AlSareah – conceptualization, data curation, investigation, methodology, project administration, writing – original draft and writing – review & editing; Hutham Tariq – conceptualization, data curation, investigation, methodology, resources, writing – original draft and writing – review & editing.
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Abstract

Introduction. Maternal iron deficiency, prevalent globally and especially in low-income regions like Basrah, Iraq, poses serious risks to maternal and fetal health. Iron deficiency in pregnancy presents with fatigue, pallor, and may be asymptomatic; diagnosis involves CBC, ferritin, iron studies, and differential exclusion.
Purpose. This study aims to determine the prevalence of iron deficiency among pregnant women, evaluate the effectiveness of current screening methods, and identify key risk factors contributing to iron deficiency in pregnancy within Basrah governorate.
Materials and methods. This cross-sectional study, conducted at Al-Basrah Teaching Hospital for the duration from the 1st of October 2024 till the 1st of October 2025. Data were collected via structured interviews, clinical exams, and lab tests (Hb and ferritin). Inclusion excluded women with hemoglobinopathies or inflammatory diseases. Iron deficiency was defined as serum ferritin ≤30 ng/ml; anemia as Hb <11 g/dL per WHO criteria.
Results. The study included 75 pregnant women, mostly under 30 years (60%) and housewives (89.3%). Overweight (41.3%) and third-trimester pregnancies (68%) were common, with only 20% taking iron supplements regularly. Moderate anemia (Hb 8–10 g/dL) was present in 49.3%, and 50.6% had serum ferritin <15 ng/mL. A significant association was found between low serum ferritin and advanced gestational age (p=0.034). Mean hemoglobin and ferritin levels decreased significantly with gestational age (p=0.05 and p=0.023, respectively).
Conclusions. A high prevalence of iron deficiency among pregnant women in Basrah, with over 85% having low serum ferritin. Contributing factors include inadequate antenatal care, poor compliance with supplementation, short inter-pregnancy intervals, and prolonged lactation.
Keywords: anemia, iron deficiency, pregnant, ferritin, hemoglobin

INTRODUCTION

Maternal health and nutrition play a crucial role in ensuring favorable pregnancy outcomes and the long-term well-being of both the mother and child. Adequate nutrition during pregnancy is essential for fetal growth, maternal physiological adaptations, and reducing the risk of pregnancy-related complications [1].

One of the most common micronutrient deficiencies in pregnancy is iron deficiency, which can lead to iron deficiency anemia (IDA), adversely affecting both maternal and neonatal health outcomes [2]. Ensuring adequate iron levels through proper dietary intake, supplementation, and early screening is fundamental in preventing adverse health consequences [3].

Iron deficiency is the most common nutritional deficiency worldwide, affecting approximately 40% of pregnant women globally, with even higher prevalence in low- and middle-income countries [4]. During pregnancy, iron requirements increase significantly due to expanded maternal blood volume, fetal growth, and placental development [2]. In many regions, particularly in the Middle East, iron deficiency remains a significant public health concern due to dietary habits, high parity rates, and limited healthcare access. Studies have shown that in Iraq, iron deficiency anemia remains prevalent among pregnant women, necessitating urgent interventions [5].

Iron deficiency during pregnancy has both immediate and long-term consequences for maternal and fetal health. In mothers, it can lead to increased fatigue, impaired immune function, cardiovascular strain, and a higher risk of perinatal infections and postpartum hemorrhage. Severe cases can also result in heart failure and increased maternal morbidity [6].

For the fetus, iron deficiency is associated with preterm birth, low birth weight, intrauterine growth restriction (IUGR), and neurodevelopmental impairments. Studies suggest that infants born to iron-deficient mothers are at higher risk of developing cognitive and behavioral deficits due to insufficient iron availability during crucial brain development stages. Given these consequences, early detection and management of iron deficiency in pregnancy are critical to ensuring optimal maternal and neonatal outcomes [2, 7].

Basrah governorate, located in southern Iraq, has a high burden of iron deficiency among pregnant women, similar to other regions in the country. Socioeconomic factors, dietary patterns, and limited healthcare access contribute to the persistence of this issue. The healthcare system in Basrah, while equipped with antenatal services, faces challenges in implementing routine screening for iron deficiency in pregnancy. Many cases go undetected due to a lack of standardized screening protocols, delayed antenatal visits, and inadequate awareness among pregnant women regarding the importance of iron supplementation [8].

AIMS OF THE STUDY

1. To determine the prevalence of iron deficiency among pregnant women.
2. To evaluate the effectiveness of current screening methods.
3. To identify risk factors associated with iron deficiency in pregnancy in Basrah governorate.

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MATERIALS AND METHODS

This is a cross-sectional study to determine the prevalence of iron deficiency among pregnant women, evaluate the effectiveness of the current screening method and identify risk factors associated with iron deficiency in pregnancy. The study was conducted in Al-Basrah Teaching Hospital for the duration from the 1st of October 2024 till the 1st of June 2025. Pregnant women who were presented to the outpatient clinic at Al-Basrah Teaching Hospital during the study period were included. Pregnant women in their first, second, and third trimesters agreed to be part of the study. While the pregnant women who have already been diagnosed with thalassemia or sickle cell disease, those who were critically ill were unable to participate in the interviews, and Women who had a diagnosis of an inflammatory condition were excluded from the study.

A questionnaire was developed for the sake of this study, the questionnaire includes the following: Socio-demographic characteristics of the patient: age, occupation residency, and educational level. The Pregnancy-related characteristics: gravity, party, any previous abortions, current pregnancy complications, gestational age, and regularity of ANC visits. Symptoms of IDA include headache, dizziness, easy fatigability, and shortness of breath. Iron-related variables: if the pregnant woman receives any iron supplementation during the pregnancy and the regularity of this supplementation, screening for the possible causes of iron deficiency anaemia includes any food shortage, poor intake, vegetarian, excessive tea and coffee consumption, warm infestation, malabsorption, short interpregnancy period, prolong lactation, and menorrhagia.

After the interview, the mother's anthropometric measurements were assessed including weight, height, and BMI. A systemic examination and blood pressure measurement was carried out. Then A 5 ml venous blood sample was drawn from each participant. 2 ml was placed in an EDTA tube for hemoglobin (Hb) testing. 3 ml was collected in a serum separator tube for serum ferritin testing. The Hemoglobin Testing (Hb): Performed using an automated hematology analyzer. The Serum Ferritin: Measured using immunoassay methods.

Diagnostic Criteria: Anemia: Defined as Hb levels <11 g/dl for pregnant women according to WHO guidelines [9]. Anemia severity was classified into three categories: mild (10–10.9 g/100 ml), moderate (7.0–9.9 g/100 ml), and severe (less than 7.0 g/100 ml). while the iron Deficiency: Defined as serum ferritin less than or equal 30 ng/ml [10].

The Basrah Health Directorate officially endorsed the conduct of this study. Informed consent was obtained from all participating patient groups.

Statistical analysis

Data analysis was conducted using the Statistical Package for Social Sciences (SPSS), version 26. Quantitative data were expressed as mean ± standard deviation, while qualitative data were summarized as frequencies and percentages. Appropriate statistical tests were applied, with the level of significance (p-value) set at ≤0.05.

RESULTS

The study includes 75 pregnant women. The majority of participants (60%) were under the age of 30 years, and a significant portion of participants (57.3%) had only primary education. Regarding their occupation, most of the women (89.3%) were housewives.

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Fifty-six percent of women had one to four children. The BMI was measured and showed that a notable proportion of women were overweight (41.3%) and 29.3% were obese. Most participants (68%) were in their third trimester. Regarding the ANC, 46.7% had irregular ANC. The women were asked about iron supplementation and only 20% were taking iron supplements regularly. All these data are presented in Table 1.

Table 1
The demographic and clinical characteristics of participants

Variables		No.	%
Age	<30	45	60.0
	30–35	22	29.3
	36–40	5	6.7
	>40	3	4.0
Education	Illiterate	16	21.3
	Primary	43	57.3
	Graduate	16	21.3
Occupation	Housewife	67	89.3
	Employer	8	10.7
Parity	Nullipara	24	32.0
	1–4	42	56.0
	≥5	9	12.0
BMI	Underweight (<18.5)	1	1.3
	Normal weight (18.5–24.9)	21	28.0
	Overweight (25–29.9)	31	41.3

Table 2 shows the Hemoglobin and Serum Ferritin Levels. Almost half (49.3%) had moderate anemia (Hb 8–10 g/dL), and 6.7% had severe anemia (Hb ≤7 g/dL). About half of the participants (50.6%) had very low serum ferritin levels (<15 ng/mL), which is a strong indicator of iron deficiency.

Table 2
The haemoglobin and serum ferritin among participants

Variables		No.	%
Hb	≥11	33	44.0
	8–10	37	49.3
	≤7	5	6.7
Serum ferittine	<15	38	50.6
	15–30	26	34.7
	>30	11	14.7

Table 3 shows the association between serum ferritin levels and sociodemographics. No significant association was found between parity and serum ferritin levels (p=0.542) There was no significant association between BMI and serum ferritin levels (p=0.142). A significant association with gestational age (p=0.034) was observed, with the lowest serum ferritin levels in the third trimester.

Table 3
The association between the serum ferritin level and participants' sociodemographics

Variables		Serum ferritin level			p-value
		<15	15–30	>30	
Age	<30	21 (55.3)	18 (69.2)	6 (54.5)	0.666
	30–35	11 (28.9)	7 (26.9)	4 (36.4)	
	36–40	4 (10.5)	0 (0.0)	1 (9.1)	
	>40	2 (5.3)	1 (3.8)	0 (0.0)	
Parity	Nullipara	9 (23.7)	10 (38.5)	5 (45.5)	0.542
	1–4	23 (60.5)	14 (53.8)	5 (45.5	
	5	6 (15.8)	2 (7.7)	1 (9.1)	
BMI	Underweight (<18.5)	0 (0.0)	0 (0.0)	1 (9.1)	0.142
	Normal weight (18.5–24.9)	7 (18.4)	10 (38.5)	4 (36.4)	
	Overweight (25–29.9)	18 (47.4)	9 (34.6)	4 (36.4)	
	Obesity class 1 (30.0–34.9)	10 (26.3)	7 (26.9)	2 (18.2)	
	Obesity class 2 (35–39.9)	3 (7.9)	0(0.0)	0 (0.0)	
Gestational age	1st trimester	4 (10.5)	5 (19.2)	2 (18.2)	0.034
	2nd trimester	2 (5.3)	8 (30.8)	3 (27.3)	
	3rd trimester	32 (84.2)	13 (50.0)	6 (54.5)	
Total		38 (100.0)	26 (100.0)	11 (100.0)	

Table 4 shows the causes of iron deficiency among participants, prolonged lactation (26.7%) and short intervals between pregnancies (25.3%) were the most common causes of iron deficiency, followed by poor dietary intake (22.7%).

Table 4
The causes of iron deficiency

Variables	No.	%
Food shortage	14	18.7
Poor intake	17	22.7
Vegetarian	4	5.3
Excessive coffee	2	2.7
Excessive tea	2	2.7
Malabsorption	5	6.7
The short interval between pregnancy	19	25.3
Prolong lactation	20	26.7

Table 5 shows the mean hemoglobin and serum ferritin levels by gestational age. Both haemoglobin and serum ferritin levels decreased significantly as gestational age advanced (p=0.05 for Hb and p=0.023 for ferritin).

Table 5
The mean Hb and mean serum ferritin concerning different gestational age

Gestational age	Hb level		p-value	S.ferttine		p-value
	mean	SD		mean	SD	
1st trimester	11.05	1.01	0.05	20.23	9.38	0.023
2nd trimester	9.89	1.27		23.46	8.01	
3rd trimester	9.61	1.05		16.13	8.79	

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DISCUSSION

Iron deficiency and iron deficiency anemia (IDA) remain significant public health concerns during pregnancy, particularly in low- and middle-income countries [11]. The physiological demands of pregnancy, combined with nutritional deficits, irregular antenatal care (ANC), and poor compliance with supplementation, often culminate in reduced hemoglobin and serum ferritin levels, increasing the risk of adverse maternal and fetal outcomes [2]. This study is especially justified in the Iraqi context, where economic challenges and healthcare access disparities may contribute to the high prevalence of maternal anemia, as previously suggested by WHO estimates [4].

An alarming 56% of the women had hemoglobin levels below the WHO threshold of 11 g/dL, with 6.7% classified as severely anemic (≤7 g/dL). Furthermore, 85.3% had low serum ferritin levels (≤30 ng/mL), indicating widespread iron deficiency, even in the absence of overt anemia. These findings are consistent with WHO global estimates that up to 50% of anemia in pregnancy is due to iron deficiency [4, 12]. The discordance between hemoglobin and ferritin levels underscores the importance of dual testing, relying on hemoglobin alone risks underdiagnosing iron deficiency. Studies from similar settings, such as Egypt and India, have echoed this concern and recommended serum ferritin testing as a routine component of ANC [13, 14].

Multiple modifiable factors were identified as contributors to iron deficiency. Prolonged lactation (26.7%), short inter-pregnancy intervals (25.3%), and poor dietary intake (22.7%) were the leading causes. These findings align with Babah et al., who demonstrated that closely spaced pregnancies and prolonged breastfeeding without supplementation significantly increase maternal iron depletion [15]. Malabsorption, vegetarianism, and excessive tea/coffee consumption were less frequently reported, but their presence is noteworthy, as these factors can impair iron absorption as reported by Lee et al. and Saboor et al. [16, 17].

Both hemoglobin and serum ferritin levels declined with advancing gestational age. Mean Hb dropped from 11.05 g/dL in the first trimester to 9.61 g/dL in the third ($p=0.05$), while serum ferritin declined from 20.23 ng/mL to 16.13 ng/mL ($p=0.023$). These findings are consistent with the known physiological decline in iron markers during pregnancy due to hemodilution and increased fetal demands [18, 19]. The statistically significant decline across trimesters confirms the inadequacy of iron supplementation and ANC practices in the study population. Moreover, only 20% of participants reported regular iron supplementation, and 44% had not taken any iron supplements during pregnancy, a striking gap in essential care.

The high prevalence of iron deficiency and suboptimal antenatal screening and supplementation practices call for urgent public health interventions in Basrah. The lack of regular ANC among 46.7% of participants and poor adherence to supplementation among 80% are critical gaps. These factors, combined with socioeconomic constraints and poor nutritional knowledge, have created a setting where maternal iron deficiency is widespread and preventable. The study supports WHO and ACOG recommendations to initiate routine iron supplementation in early pregnancy and incorporate serum ferritin testing where feasible to detect early-stage deficiency [20].

The findings from this study are in agreement with global research that demonstrates higher rates of IDA in developing countries due to poverty, dietary insufficiencies, and inadequate antenatal care [21]. Our results particularly mirror those of studies conducted

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in other Middle Eastern populations, such as Jordan, Saudia Arabia, Egypt and Iraq, where IDA prevalence among pregnant women exceeded 60% [14, 22–24].

■ CONCLUSIONS

A high prevalence of iron deficiency among pregnant women in Basrah, with over 85% having low serum ferritin. Contributing factors include inadequate antenatal care, poor compliance with supplementation, short inter-pregnancy intervals, and prolonged lactation. The following recommendations are proposed: routine serum ferritin testing, universal iron and folic acid supplementation, in parallel, and public health education campaigns to focus on strengthening antenatal care programs to expand coverage, improve adherence to supplementation protocols, and ensure equitable access to maternal nutrition services.

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Detection of Rubella Virus and The Relation of Il-10 and Il-17 Levels Among Aborted Women

Conflict of interest: nothing to declare.
Authors' contribution: Mohammed Jasim Mohammed Shallal – conceptualization, data curation, investigation, methodology, project administration, resources, writing – original draft and writing – review & editing; Hayder Raad Abbas – conceptualization, data curation, investigation, methodology, writing – original draft and writing – review & editing.
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Abstract

Introduction. Rubella is a systemic viral illness characterized by its mild nature, non-specific symptoms, infrequent diagnosis, and high transmissibility. The rubella virus readily traverses the placenta; consequently, maternal infection during pregnancy, particularly in the first trimester, poses a significant risk of fetal transmission, which may lead to complications such as miscarriage, fetal demise, and the development of congenital rubella syndrome (CRS).
Purpose. To investigate immunological markers that may be associated with rubella viral infections in aborted women, specifically cytokines IL-10 and IL-17.
Materials and methods. From October 2024 to the end of December 2024, the study included 352 participants, comprising 176 females with a history of abortions and 176 females with normal pregnancies, who acted as a control group. Serum samples were analyzed for IL-10 and IL-17 using the enzyme-linked immunosorbent assay (ELISA) method.
Results. Results of rapid tests in the abortion group and in the control group, Recent rubella virus infection according to results of rapid IgM test showed significant difference between abortion group and control group (p=0.015); in addition, old infection as proved by rapid IgG test was higher significantly in patients' group in comparison with control group (p<0.001). Comparison of titer levels of IgM and IgM for rubella virus between patients' group and control group, IgM showed no significant difference (p=0.311), but IgG was higher in a significant manner in abortion group in comparison to control group.
Conclusions. Rubella infection is associated with abortion. Bothe IL-10 and IL-17 play a significant role in the pathogenesis of abortion related to rubella viral infection.
Keywords: rubella virus, interlukein-10, interlukein-17, ELISA, abortion

■ INTRODUCTION

The rubella virus is an enveloped, positive-sense, non-segmented RNA virus classified within the genus Rubivirus, which falls under the family Matonaviridae. Prior to 2018, the rubella virus was classified within the family Togaviridae [1]. Rubella typically results

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in an acute or moderate viral illness, with transmission occurring through respiratory droplets. Following an incubation period of approximately 14 days, symptoms such as rashes, mild fever, and sore throat frequently manifest [2]. Infection with the rubella virus during early pregnancy is linked to teratogenic consequences and the potential for spontaneous abortion [3]. Interleukin is a cytokine that mediates communication among leukocytes and other cell types, displaying diverse stimulatory functions that modulate immune, inflammatory, and hematopoietic responses [4]. Interleukin-10 (IL-10) is a significant cytokine that plays a role in the reduction of inflammation. Immune cells such as macrophages, natural killer (NK) cells, dendritic cells (DCs), CD8 T cells, B cells, and dermal keratinocytes are responsible for its production [5] It modulates cellular immunity by constraining cell-mediated responses and decreasing proinflammatory cytokines during infections, thus alleviating their adverse effects. IL-10 specifically inhibits responses by diminishing antigen presentation by antigen-presenting cells (APCs) [5, 6]. The IL-17 cytokine family includes IL-17A to IL-17F. Interleukin-17 is synthesized by CD4 and Th17 cells [7]. Interleukin-17 (IL-17) is recognized as an inflammatory cytokine primarily influencing myeloid and mesenchymal cells. This process promotes the production of specific chemokines that draw neutrophils to sites of infection [5, 8]. Cytokines are crucial in mitigating the adverse effects of viral infections and pro-inflammatory responses [7, 9].

■ MATERIALS AND METHODS

Participants and study design

From October 2024 to the end of December 2024, the research was conducted. Samples were collected from Al-Rumaitha General Hospital and the Gynecology and Pediatrics Teaching Hospital in the Al-Muthanna Governorate. A total of 352 participants were included in the study, comprising 176 women who had previously experienced abortions and 176 women in a control group with normal pregnancies without abortions. All participants were between the ages of 18 and 45.

Included criteria

Categorized patients based on specific criteria. All patients participating in this study using specific data pertaining to their needs. The following patients were enrolled in the study and had their information gathered, as detailed in the questionnaire: A medical history from the family. Date of birth, place of residence, and profession were all pieces of demographic data.

Excluded criteria

The study established specific inclusion criteria for patients based on age groups, and it excluded patients 46 years old or older. The study also excluded patients with autoimmune diseases or chronic diseases.

Samples collection

This study includes 4 ml of whole blood samples from both control and patient groups. The samples are centrifuged for 5 minutes at 4000 rpm. Serum samples are then analyzed to detect virus antibodies and cytokine markers such as IL-10 and IL-17. ELISA can identify rubella through the presence of IgG and IgM antibodies.

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IL-10 and IL-17 and rubella virus IgG and IgM investigation

A human interleukin ELISA kit was used for the test. IL-10, IL-17 (Bioassay Technology Laboratory), and rubella virus IgG and IgM were measured and detected according to the ELISA kit instructions (SunLong Biotech Co., LTD).

Ethical considerations

This protocol was approved by the Board of the Research Ethics Committee of the Health Office in Al-Muthana Governorate, according to the ethical number (No. 866 on 1/8/2022).

Statistical analysis

Microsoft Office Excel 2010 and the Statistical Package for the Social Sciences (SPSS) version 23 were used for data collection, summarization, analysis, and presentation. Quantitative (numerical) variables were represented by means and SD, while qualitative (categorical) variables were represented by numbers and percentages. The Fisher exact test was used in situations where the chi-square test proved unreliable. A significance threshold was considered reached when the $P<0.05$.

■ RESULTS

Results of rapid tests in abortion group and in control group are shown in table 1. Recent rubella virus infection according to results of rapid IgM test showed significant difference between abortion group and control group (p=0.015); in addition, old infection as proved by rapid IgG test was higher significantly in patients' group in comparison with control group (p<0.001).

Table 1
Rapid tests in abortion group and in control group.

Characteristic	Abortion group, n=176	Control group, n=176	p
Rapid IgM RV			
Positive	7 (4.0%)	0 (0.0%)	0.015 F *
Negative	169 (96.0%)	176 (100.0%)	
Rapid IgG RV			
Positive	163 (92.6%)	27 (15.3%)	<0.001 C ***
Negative	13 (7.4%)	149 (84.7%)	

Notes: C – chi-square test; F – Fischer exact test; NS – not significant; * significant at p≤0.05; *** p≤0.001.

Comparison of titer levels of IgM and IgM for rubella viruses between patients' group and control group is shown in table 2 and figures 1, 2. Regarding rubella virus, IgM showed no significant difference (p=0.311), but IgG was higher in a significant manner in abortion group in comparison to control group (p<0.001).

Comparison of serum IL-10 and IL-17 levels between the abortion and control groups is shown in Table 3 and Figures 3, 4. The serum level of IL-10 was significantly lower in women with abortions compared to the control group, with a median of 216.02 pg/ml

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Table 2
Comparison of titer levels of IgM and IgM for rubella virus between patients' group and control group

Characteristic	Abortion group, n=176	Control group, n=176	p
Titer IgM RV			
Median (IQR)	14.34 (5.6)	13.94 (5.97)	0.311 M NS
Range	2.74–105.49	5.06–40.55	
Titer IgG RV			
Median (IQR)	19 (10.94)	10.35 (6.2)	<0.001 M ***
Range	0.08–508.21	3.01–34.11	

Notes: IQR – inter-quartile range; M – Mann – Whitney U test; NS – not significant; *** p≤0.001.

versus 329.79 pg/ml, respectively (p<0.001). The serum level of IL-17 was also significantly higher in women with abortions compared to the control group, with a median of 85.24 pg/ml versus 51.76 pg/ml, respectively (p<0.001).

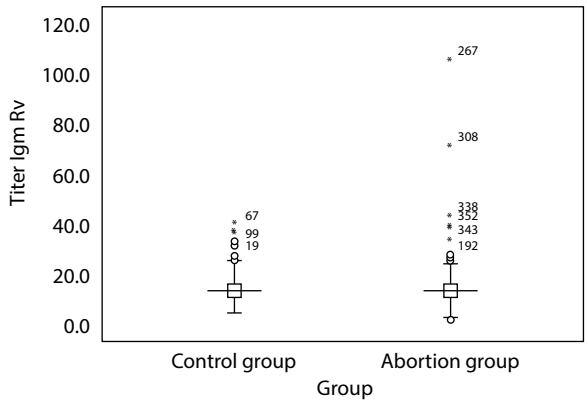

Fig. 1. Box plot showing comparison of IgM titles level of rubella virus between abortion group and control group

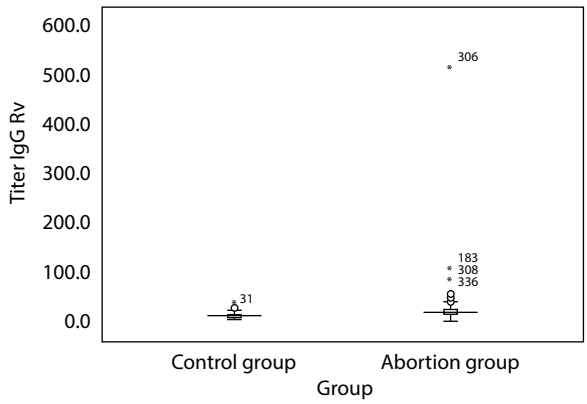

Fig. 2. Box plot showing comparison of IgG titles level of rubella virus between abortion group and control group

Table 3
Comparison of serum IL-10 and IL-17 levels between abortion and control groups

Characteristic	Abortion group, n=176	Control group, n=176	p
IL-10			
Median (IQR)	216.02 (65.55)	329.79 (323.88)	<0.001 M ***
Range	101.91–504.65	18.54–7858.60	
IL-17			
Median (IQR)	85.24 (52.37)	51.76 (19.45)	<0.001 M ***
Range	9.52–1239.57	11.29–950.62	

Notes: IQR – inter-quartile range; IL-10 – interleukin-10; IL-17 – interleukin-17; M – Mann – Whitney U test; *** significant at p≤0.001.

Correlation of IL-10 and IL-17 to Rubella infection is shown in table 4. In the control group, Rubella IgM and IgG showed no significant correlation to IL-10 (p>0.05) and significant positive correlation to IL-17 (p<0.05). In abortion group, rubella IgM showed no significant correlation to IL-10 and IL-17 (p>0.05); IgG showed no significant correlation to IL-10 (p>0.05), but significant positive correlation to IL-17 (p=0.044).

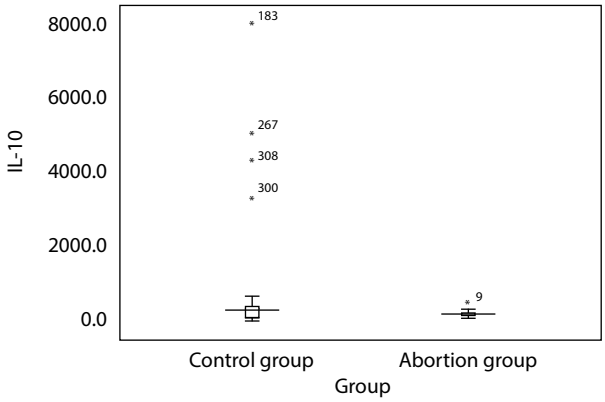

Fig. 3. Box plot showing comparison of serum IL-10 levels between abortion and control groups

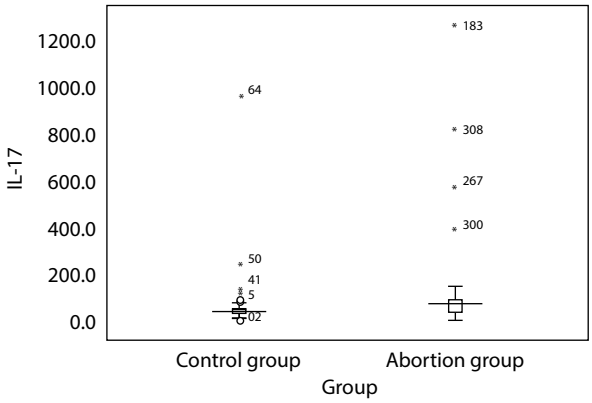

Fig. 4. Box plot showing comparison of serum IL-17 levels between abortion and control groups

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Table 4
Correlation of IL-10 and IL-17 to rubella infection

Infection	Statistic index	Control group		Abortion group	
		IL-10	IL-17	IL-10	IL-17
Rubella IgM	r	0.044	0.201	–0.002	–0.102
	p	0.558	0.007 **	0.984	0.180
Rubella IgG	r	0.008	0.183	–0.123	0.152
	p	0.911	0.015 *	0.104	0.044 *

Notes: * significant at p≤0.05; ** significant at p≤0.01; *** significant at p≤0.001.

■ DISCUSSION

Old infection with Rubella has been shown to be associated with abortion in the current study. Indeed, this finding is similar to the finding of several previous authors [10–12]. According to [13], the level of IL-10 is reduced in cases of abortion. Human IL-10, which has a variable immunologic function that can be either stimulatory or counter-regulatory, is located on chromosome 1q32. IL-10 is referred to as a Th2 cytokine and has anti-inflammatory effects against the cytokines produced by the Th1 subset [13]. Reports have also linked reduced IL-10 levels to premature birth [14]. Based on the observation of [15], median levels of IL-10 are statistically significantly lower in pathological conditions compared to matching gestational ages of normal pregnancy. Successful pregnancy depends on the mother's immune system's ability to undergo immunoregulation to tolerate the fetus and to create and sustain a nurturing environment throughout all stages of pregnancy. Several reports indicate that interleukin 10 (IL-10) is vital for normal pregnancy, and low IL-10 levels are associated with pregnancy complications [15]. A study conducted by Sharief et al. (2014) showed that serum IL-10 levels are reduced in cases of abortion [16]. On the contrary, Bakir et al. [17] proved that IL-10 was higher in control group than in recurrent spontaneous [17]. IL-17 expression has been evaluated in various pregnancy-related situations, although the data from these studies are often controversial. For instance, one study reported higher IL-17 levels in patients with unexplained recurrent spontaneous abortion (URSA) compared to women with normal early pregnancies. Importantly, in this study, samples were obtained before artificial miscarriage [18]. Conversely, Hosseini et al. [19] found high IL-17 levels in the menstrual blood of healthy fertile women but not in URSA patients, suggesting that high IL-17 levels may create an optimal environment for successful embryo implantation [19]. Additionally, the invasion of maternal tissues by the fetus can be compared to an allograft, and Th17 cells have been reported to play a significant role in allograft rejection [20]. The proposal of a Th1/Th2 balance to a favorable pregnancy outcome, in which a Th2-type cytokine response is predominant, was reinforced by a Th1 prevalence in various pregnancy complications. However, this dichotomy has been challenged by recent findings regarding the role of Th17 cytokines both in normal and pathological pregnancies [21, 22].

■ CONCLUSION

Rubella virus infection during pregnancy can lead to serious outcomes, including abortion, due to its impact on the maternal immune response. Cytokines such as IL-10 and IL-17 are key players in this process. IL-10 may facilitate viral persistence by dampening immune responses, while IL-17 contributes to inflammation that can damage

the maternal-fetal interface. Understanding the roles of these cytokines provides insight into the immunopathogenesis of rubella-related pregnancy complications and may guide future therapeutic strategies.

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Estrogen Hormone Enhances the Immune Response Against COVID-19 Infection

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Abstract

Introduction. According to data published in many previously researches, Males are more likely than females to have COVID-19 and it can be more severe.
Purpose. To indicated the impact and mechanism of action of estrogen on COVID-19 according to gender.
Materials and methods. The Google scholar, Pub Med, Research gate, and some journals specializing in viruses and hormones were searched, and some research was read and the study was conducted to compare with those studies.
Results. The larger prevalence of COVID-19 in males may be explained by a number of factors, including lifestyle choices and the different outlines of comorbidity between the genders, but it is important to note that male and female's immune systems differ. It has been demonstrated that sex variations have a significant impact on immunity reaction influential, which results to varied pathogenesis in transmittable disorder. In COVID-19, female show a further efficient innate and adaptive immune retort to viral infection than male. Since there is an increasing data indicate that estrogen has an influence on immunity retort modulation, based on previous research that studied this topic, which was collected from previous research.
Conclusions. Estradiol exerts both local and systemic protective effects against viral infections like COVID-19. Generally, the reducing of proinflammation cytokines and viral replication caused by increase of estrogen decrease the severity symptoms of COVID-19. On the other hand, the activation of immune cells caused by increase of estrogen could work against the virus and kill them faster, so the symptoms of COVID-19 are more sever in the male than female leading to more mortality and mortality in male than female.
Keywords: COVID-19, estrogen, SARS-CoV-2r, Severe acute respiratory syndrome coronavirus-2, estradiol

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■ INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) continues to increase fast over the world. According to previous studies, COVID-19 distribution differs from person to person based on their age, blood group, weight, and another factors. Moreover, it is generally known that males are more likely to have COVID-19 than females. Extensive medical and public health implications are responsible for this gap case-fatality such as smoking, alcohol consumption and circadian misalignment which are more frequent in males. Therefore, these practices may render men's lung and other organs more vulnerable to injury [1]. As a result, men may be more likely to experience conditions like hypertension, heart disease, and chronic lung diseases [2]. On the other hand, females are typically less susceptible to viral infection than males because of their more effective immune systems after sickness, which have been proven in various studies [3]. Furthermore, it has been reported that male and female have distinct natural detection and adaptive immune response against viral infections [4]. Because estrogen has a key function in women immune system, this article aims to review many studies related to the role of estrogen in the infection and development of COVID-19 infection.

COVID-19 Pathogenesis

SARS-CoV-2 is a single strand RNA virus, and has genetic traits that are notably different from SARS-CoV and MERS-CoV [5]. Virus particle invades nasal goblet cell, alveolar type II cell, and intestinal epithelial cell, generating a cascade of immune response and cytokine storms throughout the body. SARS-CoV-2's protein connect with the membrane and subsequently trigger viral RNA via adhesion to the host cells through the angiotensin-converting enzyme type 2 (ACE-2) receptor, which is uttered by pneumocyte cell, and causes ACE-2 levels to drop, ACE 2 is in charge of converting angiotensin II (Ang II) into vasodilatory and immune-suppressing. Angiotensin II link to type 1 angiotensin receptors (AT1Rs) in the lungs and activates the nuclear factor kB (NF-kB) pathway, which promotes cytokine production, causing constriction of blood vessels and inflammation [6]. Increased lung vascular permeability is caused by low levels of ACE-2 and high levels of Ang II, which leads in inflammatory injure to pulmonary tissue [7].

The cytokine storm that results since an unregulated inflammatory restraint is thought to be the major cause of cruel COVID-19, resulting pulmonary tissue destruction in a number of patients, requiring assisted breathing in some instances, and ultimately in mortality in a significant number of cases, in addition SARSCoV-2 likely stimulates mast cells in the submucosa of the respiratory system, resulting in the production of proinflammatory cytokines like interleukin (IL)-1 [8]. Also, the ACE-ANG IIAT1R pathway causes pulmonary vasoconstriction and microvascular injury, ensuing in larger and "permeable" pulmonary microvessels and injured alveoli overloaded with plasma proteins [9]. Interstitial pneumonia and pulmonary fibrosis are common complications of SARS-CoV-2 disease in the lungs. SARS-CoV-2 can also reach to additional tissues due to ACE2's widespread tissue expression, leading in multi organ collapse with vasoocclusion, increased thrombosis, and destruction of essential processes [10], Toll-like receptor (TLR3, TLR7, TLR8), retinoic acid-induced gene protein I (RIG-I), and melanoma differentiation-associated gene 5 (MDA5) identify viral RNA following it enters the host cells and stimulate a series of inflammatory cytokines [11].

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As a result of the contact between viruses and host cell, the immunity system produces a multiplicity of immunological medium to combat attacking viruses. IL-1, IL-2, IL-7, IL-12, IL-13, IL-17, GCSF, MCSF, IP-10, MCP-1, MIP-1a, HGF, IFN-c, and TNF-α are a number of the plasma cytokines and chemokines that appearance to be elevated in COVID-19 patient [12]. Neutrophil concentration, D-dimer, urea, and creatinine levels all rise dramatically in perilously sick patient, whereas lymphocyte numbers fall, Moreover, inflammatory parameters such as IL-6 and TNF-a are increased [13]. Those admitted to intensive care units (ICUs) have greater of IL-2, IL-7, IL-10, GCSF, IP-10, MCP-1, MIP-1a, and TNF-a than patient with minor infection [14].

Estrogen

Estrogen is a female steroid hormone produced by ovaries to regulate the monthly cycle in females, making it essential for women reproduction. Estrogen is vital in women and men reproduction and a variety of further systems in both men and women, include the nervous system, skeletal system and immune system. It preserves secondary sexual traits and affects the coordinated preovulatory release of gonadotropins by acting as a feedback mechanism on the hypothalamic-pituitary unit. Furthermore, estrogen is a potential autocrine regulator, with an important organizing role at its creation site [15].

Mechanism action of estrogen in SARS-CoV infection

Viral infections require a variety of innate and adaptive immune components. Immune system molecules and cells participate in a number of ways in the pathways that enable viral clearance. It is critical to investigate how immune system modulation may be related to COVID-19 infections since hormones affect immunological function. Thus, the majority of the findings in the literature discuss estradiol's immune system-boosting effects to explain the reason of increased of COVID-19 infection and its severity in the males more than females although inflammation disparities between men and women have been widely established and it has been suggested that this differences between the gender could related to a variety of causes. One of these causes, the X chromosomes code, the majority of immunity regulating genes, which ensues a typically higher immunity reaction in females, but this gender variation in inflammatory reaction, is thought to be mostly mediated by sex hormones [16]. Second cause is assumed Estrogen hormone which has a complicated function in immunity system modulation, typically in a dose-dependent manner, and it has been demonstrated to have an anti-inflammatory impact in premenopausal females at usual physiological manner [17]. Estrogens can also cause the local immune system through the activation of several cells, including; phagocytes, dendritic cells, natural killer cells, and CD8+ T cells, these cells will be combat the illness by eliminating the microbes [18].

After viral infection, the neutrophils, macrophages, and monocytes are activated and resulting in the generation of proinflammatory cytokines. However, Estradiol contributes to the prevention of inflammation by suppressing MCP-1, reducing leukocyte adhesion and migration, and preventing NF-B macrophage activation through miR-125b, which is thought to lessen the release of pro-inflammatory cytokines by macrophages (Kovats, 2015). Thus, after SARS-CoV2, proinflammatory cytokines and chemokines (such IL-6, CCL-2, and CXCL-1) increased in the men more than females [19].

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Estradiol is also known to polarize the Th2 profile through controlling B cell activity. The fact that females are more likely to produce stronger Th2 responses is a double-edged sword since it renders them more sensitive to autoimmune illnesses while simultaneously making them less susceptible to infections. Women have a greater immune response to viral infections in this aspect, which may translate to higher blood antibody concentrations following vaccination, providing superior protection [20].

As mention before after viral infection, the producing of proinflammation cytokines is increased due to the increasing in number of monocytes, macrophage and neutrophils which lead to activate lymphocytes and alveolar macrophages. The activate lymphocyte and alveolar macrophages increase the production of type I Interferon (IFN), simply chemicals that interfere with the viral life cycle, which lead to antiviral response activation. Interestingly, it has been demonstrated that the receptor α of estradiol and α and γ of IFN positively regulate one another. This result is significant since type I IFN is crucial for antiviral responses and estradiol may suppress viral replication in COVID-19 infection by reducing IFN via the ER [21]. Moreover, IFN- has been shown to promote switch class change, humoral immunity, antibody generation, and dendritic cell maturation, so the development of autoantibodies against the viral may impair the antiviral function of IFNs. According to a research, IFN-autoantibodies were found in 101 of 987 patients with life-threatening COVID-19; this occurrence is more common in males and gets worse with age [21]. However, IFN-levels were consistent throughout the infection, according to mouse research [18].

According to studies, adult women can develop more IgG and neutralizing antibodies in response to viral illnesses like the flu. In cases of COVID-19, women seem to respond immunologically better and may neutralize the virus more quickly than males. The difference in SARS-CoV-2 antibody levels between male and female patients may be a major factor in the disease's various manifestations. The B cell activation of IFN-α may be enhanced by estradiol. On the other hand, as mention before, estradiol increases IFN I production which lead to enhance the expression of TLR-7on B cells, important for pathogens recognition, in female more than male and increase these cells' ability to recognize SARS-CoV-2 in female more than male [22].

Estradiol also affects dendritic cells (DC). Estradiol boosts DC numbers during inflammation in an ER-dependent manner. It is generally known that plasmacytoid DCs have a role in antiviral responses. They have TLR9 expression, which is important for identifying viral particles. Following viral infections, TLR9 is released up to 100–1000 times more than IFN- as other blood cells [23]. According to reports, dendritic cells play a significant role in the progression of illness in a number of infections. They can deliver antigen presentation to T cells, but they can also control those cells' responses, polarizing T helper (Th) cells and priming naive T cells [24], so the development of dendritic cells in COVID-19 is a factor in the course of the illness because of their critical functions in the immune response.

Since CD8+ T have the ability to kill virus-infected cells, cytotoxic CD8+ T lymphocytes are crucial components of the immune response against intracellular infections. Estradiol may boost the activity of CD4+ cells that are specific to the virus. In a model of the influenza virus [25] studied the effects of estradiol. They discovered that estradiol has a protective effect by raising the number of CD8+ T cells, as well as, virus-specific CD4+ lymphocytes. According to report, a predictive factor linked to death in COVID-19 patients is a decrease

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in CD4+ and CD8+ T lymphocytes. This decrease has been found to be more pronounced in severe COVID-19 patients compared to those who had minor symptoms [26].

Interestingly, several animal research back up estrogen's anti-inflammatory impact relation to COVID and they found tow facts: First, male mice that infected with SARS-CoV by researcher showed larger virus titers increased pulmonary vascular permeability, and more inflammatory monocytes and macrophages in their lungs than female mice of the same age; secondly, female mice impure with SARS-CoV who were estrogens exhausted by oophorectomy or an estrogen receptors blocker had a poorer SARS-CoV prediction [27].

Other studies revealed that estrogens had a direct potent anti-inflammatory effect by its influence on the upper respiratory system and nose and improving the local immunological reaction. One of the systems Estradiol's protective properties include an increase in when nasal mucus is made, mucins and electrolytes are included, lysozyme, lactoferrin, IgA and IgG, and oligosaccharides. These substances are recognized as antiviral and antibacterial properties which would bring into play fundamental methods of prevention for upper respiratory tract infections [28].

Secondly, estrogen is able to keep mice from viral disease by straight inhibiting SARS-CoV multiplication via modulating cells metabolism. To explain that we should know that after an influenza virus, female mice given elevated doses of estrogen and decreased cytokine creation in the lung had higher endurance rates of survival [29]. These mature female mice have a greater innate immunity reaction and an additional responsive influenza-specific antibody retort than male mice due to the fact that their lungs have further virus-specific memories T cell which keep them against new viral attack [30]. Furthermore, estrogen can both suppress influenza virus's reproduction and control the estrogen receptors signaling pathway in nose epithelial cell. Estrogen is able to increase metabolic performance and assist preserve cells veracity during genetic change [31]. Also, gonadectomy or antiandrogens treatment had no effect on death in male mice impure with SARS-CoV, suggestive of that deficient in of androgen had no effect on the immunological reaction [32].

■ CONCLUSIONS

It is well known that the symptoms of COVID-19 ranging from light to severe and it is more sever in the male than female leading to more mortality and mortality in male than female. Despite the description of infection mechanism is well known now, but many questions still remain. One of these questions was about concerns about the estrogen, which has known role in enhancement of immune response, mechanism in the COVID-19 infection which has not yet been fully elucidated. This review provides several insights into its action as a following:

Estradiol has local and general roles in the body. Estradiol can activate B cells and cause the formation of neutralizing antibodies. It can also boost dendritic cells, enhance NK cytotoxicity at low doses, and increase the number of CD8+ T lymphocytes that are specific to a certain virus. On the other hand, it decreases the proinflammation cytokines produced form macrophage.

Locally, the increase of influenza-specific antibody due to the increase virus-specific memories T cell in the lungs because of increase of estrogen thus keep them against new viral attack. Furthermore, estrogen can both suppress influenza virus's reproduction and

control the estrogen receptors signaling pathway in nose epithelial cell by increase IFN I, which it interferes with the viral life cycle, via the ER. Estrogen is also able to increase metabolic performance and assist preserve cells veracity during genetic change. One of the systems Estradiol's protective properties include an increase in when nasal mucus is made, mucins and electrolytes are included, lysozyme, lactoferrin, IgA and IgG, and oligosaccharides. These substances are recognized as antiviral and antibacterial properties which would bring into play fundamental methods of prevention for upper respiratory tract infections

Generally, the reducing of proinflammation cytokines and viral replication caused by increase of estrogen decrease the severity symptoms of COVID-19. On the other hand, the activation of immune cells mentioned before caused by increase of estrogen could work against the virus and kill them faster.

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Accuracy of Transvaginal Ultrasound in the Diagnosis of Adenomyosis in Comparison with MRI

Conflict of interest: nothing to declare.
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Abstract

Introduction. Adenomyosis is a benign uterine disease characterized by the presence of ectopic endometrial glands and stroma surrounded by a hypertrophic myometrium. Traditionally, diagnosis is possible only through histological examination after hysterectomy. However, advancements in medical imaging techniques, specifically magnetic resonance imaging (MRI) and transvaginal ultrasonography (TVS), have revolutionized noninvasive diagnostic approaches.
Purpose. To evaluate the diagnostic accuracy of TVUS compared with MRI as a standard diagnostic method.
Matrrials and methods. This prospective diagnostic accuracy study was conducted at an outpatient clinic during the period from 1st of March 2024 to 1st of March 2025. All women who were referred from the gynecological outpatient clinic for radiology with clinical symptoms suggestive of adenomyosis for further radiological assessment were included. The findings from the transvaginal ultrasound examination were assessed and tabulated along with the results of the MRI scans.
Results. In total, 100 women were included. The mean age was 42.3 years, 57% resided in urban areas, and 62% were multiparous. The presenting symptoms included pelvic pain (68%) and dysmenorrhea (87%) with a mean symptom duration of 11.9 months. The TVUS and MRI findings were compared for diagnostic accuracy. TVUS demonstrated a sensitivity of 81.17%, specificity of 80%, positive predictive value (PPV) of 95.83%, negative predictive value (NPV) of 42.86%, and an overall agreement of 81%.
Conclusion. This study demonstrates that TVUS is a sensitive, specific, and reliable first-line imaging modality for adenomyosis diagnosis. It offers high PPV and reasonable accuracy, especially when key features, such as heterogeneous myometrium and myometrial cysts, are present. The study limitations include the lack of histopathological confirmation of diagnosis, small sample size, and single center, which may limit the generalizability of the results. The study did not provide information on the inter-rater reliability or agreement between radiologists and/or sonographers.
Keywords: accuracy, transvaginal ultrasound, MRI, hysterectomy, adenomyosis

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■ INTRODUCTION

Adenomyosis is a benign uterine disease characterized by the presence of ectopic endometrial glands and stroma surrounded by a hypertrophic myometrium. This leads to symptoms such as heavy menstrual bleeding and pelvic pain. Traditionally, diagnosis is only possible through histological examination after hysterectomy [1]. However, advancements in medical imaging techniques, specifically magnetic resonance imaging (MRI) and transvaginal ultrasonography (TVS), have revolutionized noninvasive diagnostic approaches [2, 3].

The pathogenic mechanisms involved in adenomyosis need to be fully elucidated; however, in the last decade, an increasing number of studies have shown that sex steroid hormone receptors, inflammatory molecules, extracellular matrix enzymes, growth factors, and neuroangiogenic factors play a major role [4].

MRI has been used as a diagnostic tool for adenomyosis, as the increased thickness of the junctional zone (JZ) with a suggested cut-off of 12 mm and the presence of myometrial cysts are common criteria [5]. A principal limitation of MRI is the absence of a definable junctional zone on imaging, which occurs in 20% of premenopausal women [5]. Later studies suggested additional features, such as an irregular appearance of the JZ and its relationship with the thickness of the entire myometrium. Another diagnostic feature is the presence of small punctate cystic foci within the JZ. The diagnosis of adenomyosis by MRI has been reported to be highly reproducible, with high inter-rater agreement for various JZ measurements [6].

Transvaginal ultrasound (TVS), on the other hand, utilizes two-dimensional (2D) and three-dimensional (3D) imaging techniques to assess adenomyosis. The sonographic features used for diagnosing adenomyosis using TVS are well described [7]. A recent consensus paper by the Morphological Uterus Sonographic Assessment (MUSA) consortium distinguished between direct features of adenomyosis [8]. Direct features include myometrial cysts, hyperechogenic islands, and echogenic subendometrial lines and buds, indicating the presence of ectopic endometrial tissue in the myometrium [8].

JZ was poorly visualized using 2D TVS. Three-dimensional (3D) TVS offers the possibility of assessing the coronal plane and using Volume Contrast Imaging (VCI, i.e., thick slices) to improve visualization of the JZ, which facilitates the evaluation of its thickness and irregularity [9].

Reproducibility studies have shown a high level of agreement when evaluating the presence or absence of adenomyosis using TVS but less agreement when assessing different ultrasound features of the disease [10].

MRI is highly valuable in adenomyosis diagnosis, but it is not considered the absolute gold standard. Instead, a comprehensive approachinvolving medical history, clinical manifestations, and imaging studies (transvaginal ultrasound (TVUS) and MRI)is typically regarded as the most effective diagnostic strategy. However, the true gold standard and definitive diagnosis of adenomyosis is histopathological examination of the uterus after hysterectomy. MRI is highly sensitive (93%) and specific (86%) for adenomyosis, especially in distinguishing it from fibroids. This is especially useful when TVUS findings are inconclusive. It is excellent for soft tissue lesion detection, operator-independent, and useful for complex or equivocal lesions. The disadvantages are that it is more expensive, less accessible, and not always utilized when TVUS is available.

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This study is the first locality research to establish a new set of TVUS features predictive of adenomyosis with high specificity, and proposes a diagnostic model for regions where MRI access is limited. This emphasizes the requirement for TVUS as a low-cost and first-line tool and shows how trained general gynecologists (vs. radiologists) can achieve acceptable diagnostic accuracy with TVUS. Lack of time and short follow-up time with loss of follow-up of cases prevented longitudinal accuracy studies with histopathologic correlation to validate imaging accuracy against pathology. So, the aims of the study to evaluate the diagnostic accuracy of transvaginal ultrasound (TVUS) compared to magnetic resonance imaging (MRI) as a method for diagnosis.

MATERIALS AND METHODS

Study design and setting

This was a prospective diagnostic accuracy study conducted at the outpatient clinic of Bint AlHuda Teaching Hospital. The study was conducted during the period from the 1st of March 2024 to the 1st of March 2025. All women who were referred from the gynecological outpatient clinic to the radiology clinic with clinical symptoms suggestive of adenomyosis (e.g., dysmenorrhea, menorrhagia, or chronic pelvic pain) for further radiological assessment were included. A flowchart of the study is shown in Figure 1.

Ethical approval

Written informed consent was obtained from all study participants. The study conform to the 2000 Helsinki declaration-updated and was approved by the Medical Ethical Committee of College of Medicine, University of Thi-Qar (no. 7/54/676 date: 09.09.2024).

Inclusion criteria

- Women aged 18–50 years.
- Presenting with symptoms suggestive of adenomyosis.
- No previous hysterectomy or uterine surgery.
- Agree to provide informed consent.

Exclusion criteria

- Women with previous uterine surgery (e.g., myomectomy).
- Coexisting pelvic pathologies such as ovarian tumors or fibroids that obscure imaging evaluation.
- Patients with an unclear diagnosis of adenomyosis on pelvic MRI or those whose MRI could not be evaluated because of technical issues.
- Patients in which adenomyosis was indecently discovered while imaging a different pelvic condition.
- Patients who refuse to participate were excluded from the study.

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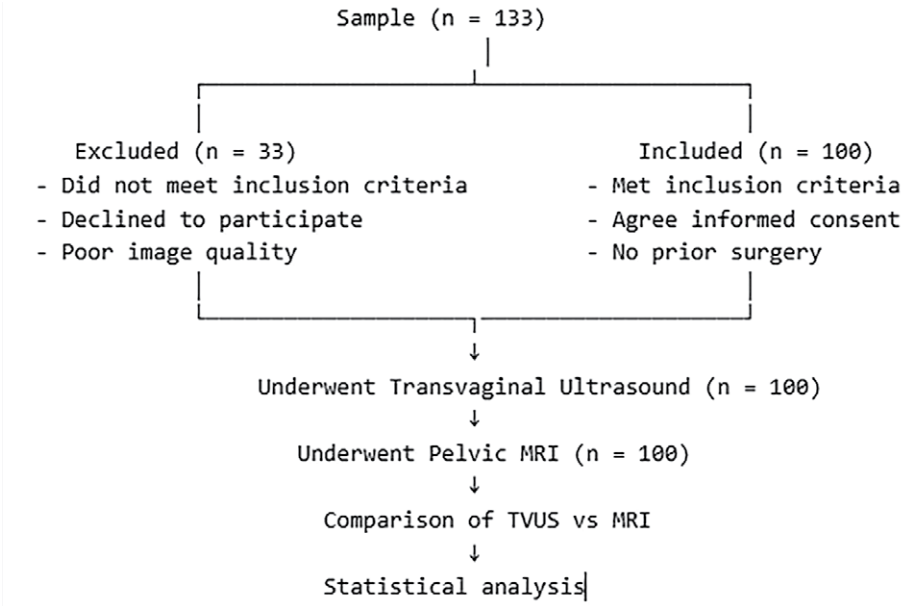

Fig. 1. Flow chart of the study

Sample size calculation

The formula for estimating sample size was:

$$n = \frac{Z2 \times P\,(1-P)}{d2}.$$

Where:

- n – sample size;
- Z – Z-score;
- P – proportion;
- d – margin of error.

Data collection

This was done using a questionnaire for the purpose of the study and included the following variables: age, residency, and socioeconomic status. Clinical characteristics included presenting symptoms (e.g., dysmenorrhea, menorrhagia, or chronic pelvic pain), symptom duration, and severity. Gynecological history included parity, menstrual history, and history of infertility. Past surgical history included any previous pelvic surgery (e.g., cesarean section or myomectomy).

Transvaginal ultrasound

The women were then sent for Transvaginal Ultrasound (TVUS) performed by Volsun E6. TVUS was performed by an experienced sonographer using a high-frequency transvaginal probe. Transvaginal ultrasound was performed using endovaginal curved array

transducers (5–12 MHz). This involved acquiring grayscale and color Doppler sonograms of the uterus, ovaries, and adnexa. The participants will be instructed to empty their bladders before the procedure. Images were captured in both longitudinal and transverse planes. The parameters that were evaluated included the bulky uterus, pseudo-widening of the junctional zone (and its measurement), ultrasound echotexture, myometrial cyst, echogenic nodule/streaky myometrium, and relative absence of mass effect. TVUS may be used to diagnose adenomyosis, including heterogeneous myometrium, myometrial cysts, asymmetrical myometrial thickening, and subendometrial echogenic linear striations.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) was performed using Syngo. via Siemens (Siemens). The MRI protocol included axial T1 weighted sequences with fat saturation, axial, sagittal, and coronal T2-weighted sequences, and T1WI post-contrast images. Slice thickness was 4–6 mm. Sequences were performed using 1.5–3 T clinical MRI systems. Parameters to Evaluate included the thickness of the junctional zone (≥12 mm is diagnostic). Myometrial spots with high signal intensity on T2-weighted images were used.

The findings from the transvaginal ultrasound examination were assessed and tabulated along with the results of the MRI scans.

All examinations were conducted by two senior radiologists (first, a consaltant and associated professor of radiology with 23 years of experience, and second, a consaltant and associated professor of radiology and interventional radiology with 20 years of experience) at the radiology department. Both TVUS and MRI operators do not know each other's findings to raise the study's innovation and reduce bias.

Statistical analysis

Data were entered using the Statistical Package for Social Sciences (SPSS) version 26. Appropriate statistical tests were performed. Results were described in the form of frequencies and percentage distributions for qualitative data and mean and SD for quantitative data. The chi-square test was used to estimate the differences in quantitative variables between the two groups. Sensitivity, specificity, PPV, NPP, and accuracy indices were calculated to measure the accuracy between the two groups. In all statistical analyses, the level of significance (p-value) was set at <0.05.

■ RESULTS

The study included 100 women who presented with signs and symptoms of adenomyosis and were sent to the radiological department for diagnosis. The mean age was 42.3 years, 57% lived in urban areas, and 62.0% were multiparous. These data are presented in Table 1.

Table 2 shows the presenting symptoms in women, 68% of whom had pelvic pain and 87% had dysmenorrhea. The mean duration of the symptoms was 11.9 months. The women were asked about their history of hormonal treatment, and 76% had a positive history.

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Table 1
The demographic distribution of patients

Variables		No.	%
Age	Mean±SD	42.3±3.61	
Residency	Rural	43	43.0
	Urban	57	57.0
Parity	Nullipara	38	38.0
	Multipara	62	62.0

Table 2
The presenting symptoms-related variables

Symptoms	No.	%
Pelvic pain	68	68.0
Dysmenorrhea	87	87.0
Menorrhagia	61	61.0
Dyspareunia	35	35.0
Duration of symptoms in months	11.9 ±2.5	
History of hormonal treatment	76	76.0

Table 3 shows the prevalence of TVUS findings; a bulky uterus was noticed among 55% of them, and 77% of them had a heterogeneous myometrium. Widening of the junctional zone was present in 62% of cases, and myometrial cysts were present in 69% of cases (Figure 2A and 3A).

Table 3
The prevalence of TVUS findings

Variables	No.	%
Bulky uterus	55	55.0
Heterogenous myometrium	77	77.0
Widening of junctional zone	62	62.0
Myometrial cyst	69	69.0
Globular uterine shape	56	56.0

Table 4 shown the prevalence of various MRI findings, the most common finding is the Widening of the junctional zone >12 mm (81%) Followed by heterogeneous myometrium with 79%. Both bulky uterus and myometrial cysts occur in 75% of cases (Figure 2B and 3B).

Table 4
The prevalence of MRI findings

Variables	No.	%
Bulky uterus	75	75.0
Heterogenous myometrium	79	79.0
Widening of junctional zone >12 mm	81	81.0
Myometrial cysts	75	75.0
Hyperintense SI in T2WI	69	69.0

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Table 5 evaluated the diagnostic performance of TVUS against MRI as the gold standard diagnostic test for adenomyosis. TVUS showed a high sensitivity of 81.17% and specificity of 80%. In addition, a high positive predictive value of 95.83% was found, suggesting a strong correlation between positive TVUS and the presence of adenomyosis. In contrast, the negative predictive value was lower at 42.86%. A total agreement of 81.0% indicated a good overall concordance ($p<0.001$).

Table 5
The sensitivity, specificity, PPV, NPV, and total agreement between the TVUS and MRI

Variables	Adenomyosis present (by MRI)	Adenomyosis absent (by MRI)	Total	*p-value
Adenomyosis present (by TVULS)	69 (TP)	3 (FP)	72	<0.001
Adenomyosis absent (by TVULS)	16 (FN)	12 (TN)	28	
Total	85	15	100	
Sensitivity of TVUS; 81.17% Specificity of TVUS; 80.0% Positive predictive value; 95.83% Negative predictive value; 42.86% Total agreement (accuracy); 81.0%				

Note: * chi-square.

Table 6 shown the diagnostic performance of the TVUS findings. The most sensitive finding was Heterogenous myometrium 84.7% followed by Myometrial cyst 76.47%. In terms of specificity, the widening of the junctional zone and a globular uterine shape show the highest value at 80.0%.

The overall accuracy ranged from 62% for a bulky uterus to 82.0% for a heterogeneous myometrium. Significant p-values indicated the important role of these parameters in the diagnosis of adenomyosis.

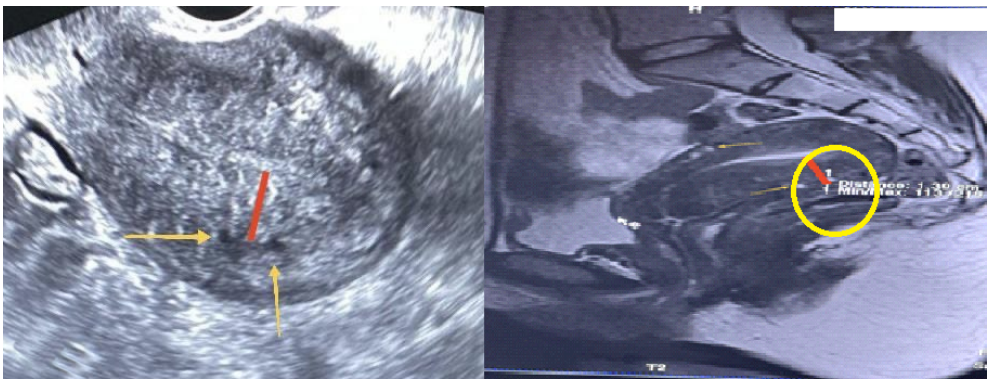
Table 6
The diagnostic performance of the TVUS findings in detecting adenomyosis

Variables	Sensitivity	Specificity	Accuracy	p-value
Bulky uterus	60.0	73.3	62.0	0.016
Heterogenous myometrium	84.7	66.7	82.0	0.001
Widening of junctional zone	69.41	80.0	71.0	0.002
Myometrial cyst	76.47	73.3	76.0	0.001
Globular uterine shape	62.35	80.0	65.0	0.002

■ DISCUSSION

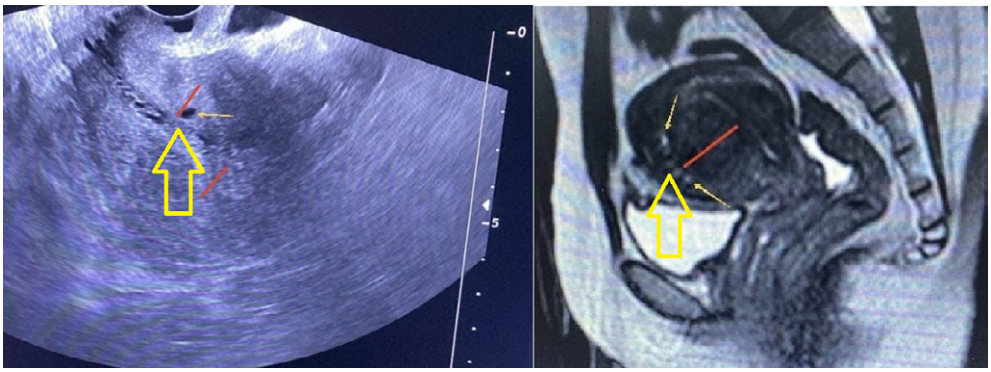
The diagnosis of adenomyosis poses a clinical challenge due to overlapping symptoms with other gynecological pathologies, such as endometriosis and fibroids [11]. Historically, adenomyosis has been diagnosed postoperatively via histopathological examination. However, advancements in imaging technologies, particularly TVUS and magnetic resonance imaging (MRI), now allow for noninvasive preoperative diagnosis with increasing accuracy [12].

The mean age of the study population was 42.3 years, which aligns with the literature indicating that adenomyosis predominantly affects women in their late reproductive years, particularly those between 40 and 50 years of age, which is in line with Pados et



A B

Fig. 2. A – Endovaginal ultrasound of 44-years-old female with heavy irregular vaginal bleeding ultrasound show widening of the pseudo junctional zone (red) hyper and hyperintense foci; B – MRI sagittal T 2 of the same patient shows a widening of junctional. Zone. 14 mm red arrow and hyperintense foci yellow arrow



A B

Fig. 3. A – Endo vaginal ultrasound of 50-years-old female presented with irregular heavy bleeding and pelvic pain. It shows a globular uterus and a widening of junctional. Zone (red line) and myometrial cyst (yellow); B – MRI sagittal T 2 of the same patient show the same finding. Junctional zone 16 mm (red) and myometrial cyst yellow

al. (2023) [13]. Most participants were multiparous (62%) and resided in urban settings (57%), which is consistent with known risk factors for adenomyosis such as high parity and increasing age, as reported by Taran et al. [14]. Notably, a significant number of women reported chronic symptoms including dysmenorrhea (87%), pelvic pain (68%), and menorrhagia (61%), reinforcing the clinical burden of the disease. The high percentage of prior hormonal therapy use (76%) suggests a history of attempted symptom management, which may reflect either recurrent disease or treatment-resistant cases, in agreement with MacGregor et al. and Bakhsh et al. [15, 16].

Table 3 and Figure 1 show that the most common TVUS findings were a heterogeneous myometrium (77%) and myometrial cysts (69%). These findings are consistent with the known sonographic hallmarks of adenomyosis described by the Morphological Uterus Sonographic Assessment (MUSA) group, which lists heterogeneous myometrial echotexture and the presence of small cystic areas as key indicators [17].

A bulky uterus was found in 55% of the cases, a classical but nonspecific finding in adenomyosis, which can also be seen in fibroids [18]. Widening of the junctional zone (62%) and globular uterine shape (56%) further supported the diagnosis, with prior studies by Tellum et al. and Dason et al. confirming these markers as part of the TVUS diagnostic spectrum [6, 19].

When comparing TVUS findings to diagnostic performance, heterogeneous myometrium emerged as the most sensitive (84.7%) and accurate (82%) feature. This mirrors the findings of Jain et al., who demonstrated that echogenic heterogeneity remains the most reliable ultrasonographic predictor of adenomyosis [20]. However, the specificity of this sign was relatively low (66.7%), indicating that although it is frequently present, it may not be exclusive to adenomyosis, necessitating correlation with other features.

The myometrial cyst had a sensitivity of 76.47% and a good specificity (73.3%), reaffirming its diagnostic value. Widening of the junctional zone showed high specificity (80%), suggesting its utility in confirming the diagnosis when present, albeit with lower sensitivity (69.41%). These findings are consistent with those of Tellum et al. [6].

MRI identified junctional zone widening >12 mm in 81% of cases, followed by heterogeneous myometrium (79%), and both myometrial cysts and bulky uterus in 75% of cases. These results are consistent with published MRI diagnostic criteria for adenomyosis. A junctional zone thickness >12 mm has been repeatedly emphasized as a sensitive and specific finding, as reported by Zhang et al. and Agostinho et al. [12, 21].

A hyperintense signal on T2-weighted images (69%) was the least frequent finding but remained an important indicator, often representing small hemorrhagic foci within ectopic endometrial tissue. Although less common, this sign can be highly specific in the right clinical context, as claimed by Bourgioti et al. [22].

MRI demonstrated a slightly higher prevalence of diagnostic features than TVUS did. This supports the consensus in the literature that MRI provides superior tissue contrast and multiplanar capability, leading to improved detection and characterization of adenomyosis, especially in subtle or coexisting pathologies, as reported by Agostinho et al. (2017) and Dueholm et al. [21, 23].

The evaluation of TVUS as a diagnostic tool compared to MRI showed a sensitivity of 81.17% and specificity of 80%, with a remarkable positive predictive value (PPV) of 95.83%. These figures are in agreement with a previous study by Cerovac et al., which reported TVUS sensitivities ranging between 72% and 86%, specificities between 81% and 85%, and a PPV of 95% when compared to MRI or histopathology [24].

The clinical impact of the low negative predictive value (NPV) of 42.86% suggests a high false-negative rate, meaning that a significant number of cases may be incorrectly classified as negative, potentially leading to missed diagnoses. The reasons are the small size and depth of the lesions, operator dependency of TVUS, limited visualization due to bowel gas, and patient movement. Furthermore, we suggest strategies to mitigate the risk of false negatives, such as adjunctive imaging, long follow-up protocols, risk

stratification models, and an increased number of radiologist operators. The relatively low negative predictive value (NPV) of 42.86% indicates that negative TVUS cannot confidently rule out the disease, and MRI should be considered when clinical suspicion remains high despite a negative ultrasound. The overall concordance rate of 81% and statistically significant p-value (<0.001) support TVUS as a valid frontline diagnostic modality, particularly in primary and secondary care settings, where MRI may not be readily available [5].

The findings of this study reinforce the value of TVUS as a highly accessible and accurate diagnostic tool for adenomyosis, especially when interpreted in conjunction with a detailed clinical history. Heterogeneous myometrium and myometrial cysts, in particular, should raise clinical suspicion and warrant further evaluation and empirical management. However, given the limitations of TVUS in ruling out adenomyosis (as reflected in the low NPV), MRI remains essential in equivocal cases or presurgical planning, and these findings agree with those of Krentel et al. [25].

TVUS is more accessible, cost-effective, and less time-consuming than MRI in the diagnosis of adenomyosis [26]. Understanding its potential contributions to surgical planning, such as identifying the extent or location of lesions, would provide a more comprehensive view of its clinical utility. Including this perspective could help clarify when TVUS alone may suffice, and when MRI is necessary as an adjunct.

■ CONCLUSIONS

This study demonstrated that TVUS is a sensitive, specific, and reliable first-line imaging modality for the diagnosis of adenomyosis. It offers high PPV and reasonable accuracy, especially when key features, such as heterogeneous myometrium and myometrial cysts, are present. However, MRI remains the gold standard, particularly in ambiguous or complex cases. The integration of both modalities, guided by clinical judgment, may offer the most comprehensive diagnostic approach for patients with suspected adenomyosis.

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Association of Short Interpregnancy Interval with Fetal Complications

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Abstract

Introduction. Interpregnancy interval (IPI), defined as the time between a previous delivery and the conception of the subsequent pregnancy, plays a vital role in maternal and fetal outcomes. Both short and long IPIs have been associated with adverse events such as preterm birth, low birth weight, and stillbirth. The World Health Organization recommends a minimum interval of 24 months for optimal outcomes.
Purpose. To assess the relationship between short interpregnancy interval (≤18 months) and fetal complications among pregnant women attending Al-Basra Maternity and Children Hospital.
Materials and methods. A prospective cohort study enrolling 200 pregnant women aged 18–48 years with singleton pregnancies and at least one previous live birth. Participants were grouped as short IPI (≤18 months, n=73) or longer IPI (>18 months, n=127). Data were obtained via structured interviews and medical records.
Results. Maternal age, education, and BMI were significantly associated with IPI ($p<0.05$). No significant differences were found for parity, delivery mode, or antenatal care. Fetal complications were significantly higher in the short IPI group: preterm birth (28.8% vs. 3.9%, $p<0.001$), low birth weight (9.6% vs. 1.6%, $p=0.008$), NICU admission (35.6% vs. 15.7%, $p=0.01$), and stillbirth (2.74% vs. 0.79%, $p=0.027$).
Conclusion. Short interpregnancy interval (<18 months) is significantly associated with adverse fetal outcomes, including preterm birth, low birth weight, NICU admission, and stillbirth. Strengthening postpartum family planning and nutritional counseling is essential to improve perinatal outcomes.
Keywords: interpregnancy interval, fetal complications, preterm birth, low birth weight, NICU admission

■ INTRODUCTION

The interpregnancy interval (IPI), defined as the duration between the end of one pregnancy and conception of the next, is a critical determinant of maternal and neonatal

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outcomes. Both excessively short and prolonged IPIs are associated with a range of complications that may affect the health of both mother and fetus. Optimal spacing allows for the restoration of maternal physiology, replenishment of nutritional reserves, and adequate recovery of reproductive function. The World Health Organization recommends an IPI of at least 24 months following a live birth to minimize risks of adverse outcomes [1].

Short IPI, commonly defined as less than 18 months, has been associated with higher rates of preterm delivery, low birth weight, small for gestational age (SGA) infants, and stillbirth [2]. These associations have been consistently observed across various populations and socioeconomic settings. In low- and middle-income countries, short birth intervals remain prevalent due to limited access to contraception, cultural preferences for larger families, early marriage, and reduced awareness of family planning options [3].

Several mechanisms have been proposed to explain the biological link between short IPI and adverse fetal outcomes. The maternal nutrient depletion hypothesis suggests that close successive pregnancies do not allow sufficient time for replenishment of key nutrients such as folate, iron, and calcium, which are essential for healthy fetal development [4]. Additionally, incomplete recovery of the endometrium, hormonal imbalances, and residual postpartum inflammation may contribute to impaired placental implantation and function [5].

Despite extensive global research, there remains a need for regional data to contextualize these findings. Differences in nutrition, healthcare accessibility, and social behavior may influence the relationship between IPI and perinatal outcomes. In Iraq, limited studies have explored this association. Therefore, this study was designed to evaluate the relationship between short interpregnancy interval (≤18 months) and fetal complications among pregnant women attending Al-Basra Maternity and Children Hospital.

■ MATERIALS AND METHODS

This prospective cohort study was conducted at the Obstetrics and Gynecology Department, Al-Basra Maternity and Children Hospital, from September 2024 to September 2025. A total of 200 pregnant women aged 18–48 years with singleton pregnancies and at least one previous live birth was enrolled. Participants were divided into two groups according to their interpregnancy interval: short IPI (≤18 months, n=73) and longer IPI (>18 months, n=127).

Women with chronic medical disorders such as hypertension or diabetes, multiple gestations, or known fetal anomalies were excluded. Data were collected using structured interviews and verified with medical records. Variables included maternal age, parity, body mass index (BMI), educational level, employment status, mode of delivery, antenatal care attendance, and obstetric history.

Fetal outcomes evaluated were gestational age at delivery, birth weight, neonatal intensive care unit (NICU) admission, and stillbirth. Ethical approval was obtained from the Scientific Council, College of Medicine, University of Basrah, and verbal informed consent was obtained from each participant.

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 26.5. Categorical data were expressed as frequencies and percentages and compared using the chi-square test. A p-value less than 0.05 was considered statistically significant.

■ RESULTS

The study included 200 women, divided into two groups based on interpregnancy interval: 127 women with an interval of more than 18 months and 73 women with a shorter interval of less than 18 months. Maternal age, educational level, and BMI were significantly associated with IPI. Employment status showed no significant difference (p=0.224) (Table 1).

Table 1
Maternal characteristics in relation to interpregnancy interval

Characteristics		Interpregnancy Interval		P-value
		Not short (more than 18 months), N=127	Short (Less than 18 months), N=73	
Maternal Age (years)	<20	0 (0%)	7 (9.6%)	0.001
	20 – <30	78 (61.4%)	50 (68.5%)	
	30 – <40	41 (32.3%)	14 (19.2%)	
	≥40	8 (6.3%)	2 (2.7%)	
Mother's Education (years)	Primary or less	25 (19.69%)	24 (32.9%)	<0.01
	Secondary school	75 (59%)	43 (58.9%)	
	Bachelor's or higher	27 (21.26%)	6 (8.2%)	
Employment Status	Unemployed (Housewife)	123 (96.9%)	68 (93.2%)	0.224
	Employed	4 (3.1%)	5 (6.8%)	
BMI (kg/m^2)	Underweight (<18.5)	0 (0%)	1 (1.4%)	0.014
	Normal (18.5–24.9)	40 (31.5%)	38 (52.1%)	
	Overweight (25–29.9)	61 (48%)	25 (34.2%)	
	Obese (≥30)	26 (20.5%)	9 (12.3%)	

No significant association was observed between IPI and maternal complications, mode of delivery, parity, or antenatal care attendance (Table 2).

Table 2
Maternal health status in relation to interpregnancy interval

Characteristics		Interpregnancy Interval		P-value
		Not short (more than 18 months), N=127	Short (Less than 18 months), N=73	
Complications in Current Pregnancy (HT, DM)	Present	18 (14.2%)	7 (9.6%)	0.345
	Absent	109 (85.8%)	66 (90.4%)	
Mode of Delivery	NVD	98 (77.2%)	49 (67.1%)	0.121
	CS	29 (22.8%)	24 (32.9%)	
Parity	1	31 (24.4%)	24 (32.9%)	0.371
	2	40 (31.5%)	18 (24.7%)	
	≥3	56 (44.1%)	31 (42.5%)	
Number of ANC Visits	<4	18 (14.2%)	7 (9.6%)	0.345
	≥4	109 (85.8%)	66 (90.4%)	

Short IPI was significantly associated with increased rates of preterm birth, low birth weight, NICU admission, and stillbirth (Table 3).

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Table 3
Pregnancy outcomes to interpregnancy interval

Pregnancy Outcomes		Not short (more than 18 months), N=127	Short (Less than 18 months), N=73	P-value
Birth timing	Preterm	5 (3.9%)	21 (28.8%)	<0.001
	Term	122 (96.1%)	52 (71.2%)	
Birthweight	(<2.5 kg	2 (1.6%)	7 (9.6%)	0.008
	=>2.5 kg	125 (98.4%)	66 (90.4%)	
Perinatal outcome	Alive	126 (99.21%)	71 (97.26%)	0.027
	Still birth	1 (0.79 %)	2 (2.74%)	
NICU Admission	Present	20 (15.7%)	26 (35.6%)	0.01
	Absent	107 (84.3%)	47 (64.4%)	

■ DISCUSSION

The findings of this study demonstrate that a short interpregnancy interval of less than 18 months significantly increases the risk of adverse fetal outcomes, including preterm delivery, low birth weight, NICU admission, and stillbirth. These results are consistent with multiple previous studies that have identified short birth spacing as a preventable contributor to poor perinatal outcomes [6].

The higher prevalence of short IPI among younger and less educated women reflects the influence of sociodemographic factors on reproductive behavior, as previously reported in studies from Spain and the United States [7]. Women with limited education may have reduced access to contraception and lower health literacy, contributing to inadequate birth spacing [8].

The mechanisms linking short IPI to adverse fetal outcomes are multifactorial. The maternal nutrient depletion hypothesis provides a key explanation – closely spaced pregnancies may not allow sufficient time for replenishment of essential nutrients such as folate and iron, which are critical for fetal growth and placental function [9]. Moreover, incomplete uterine recovery and residual inflammatory processes following the prior pregnancy can negatively affect placental implantation and perfusion, increasing the likelihood of preterm birth and intrauterine growth restriction [10].

The present study supports international evidence, including meta-analyses by Wen et al. and Ni et al., showing that both very short (<6 months) and excessively long (≥60 months) IPIs are linked to higher risks of adverse outcomes, producing a U-shaped relationship between spacing and risk [11]. Similar trends have been observed in regional studies from Jordan, Oman, and Ethiopia, where short IPI was associated with higher rates of preterm birth and low birth weight [12].

Although the association between IPI and maternal complications such as hypertension and diabetes was not significant in this study, other research has shown variable results depending on population characteristics [13]. The lack of statistical significance here may reflect the relatively small sample size and exclusion of high-risk pregnancies.

The findings underscore the importance of implementing effective postpartum family planning and nutritional education to prevent closely spaced pregnancies. The WHO recommends a minimum spacing of 24 months, while ACOG and SMFM suggest an optimal interval of 18–24 months to improve outcomes [14]. Integration of counseling into antenatal and postnatal care services can help reduce preventable fetal complications [15].

■ CONCLUSION

Short interpregnancy interval (<18 months) is strongly associated with fetal complications, particularly preterm birth, low birth weight, NICU admission, and stillbirth. Younger age, lower education, and normal-to-low BMI were predictors of short IPI.

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