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Evaluation Of The Impact Of Paternal Sperm DNA Quality And Maternal Follicular And Hormonal Parameters On Intrauterine Insemination (IUI) Outcomes In Infertile Couples In Kirkuk City, Iraq

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Abstract
The success of intrauterine insemination (IUI) depends on several factors, including endocrine function, hormonal balance, the number and size of the oocytes in the wife, and the sperm quality of the husband. Therefore, evaluating infertile couples before initiating treatment is essential to avoid unnecessary procedure
Aim of Study:*The present study aimed to investigate the impact of paternal sperm DNA quality, follicular parameters such as the number and size of follicles on the day of insemination, and maternal hormonal factors including FSH, LH, progesterone, and AMH on IUI outcomes among some infertile couples, in order to improve IUI management in the community of Kirkuk, Iraq, where such research remains limited.*
Methods: *The present study included* ***96*** *infertile couples during the period from December 2023 to February 2025. Paternal sperm DNA quality, as well as maternal follicular and hormonal parameters, were assessed. Appropriate statistical analysis tools were applied*
Results: *The results showed significant differences in paternal DNA fragmentation, oocyte size, and FSH levels across all age groups in couples who responded to intrauterine insemination (IUI) compared to non-responders. However, no significant differences were observed in AMH levels or the number of oocytes across all age groups.*
Conclusion: *The pregnancy rate decreases with the reduction in oocyte size and FSH levels, which negatively affects the response to intrauterine insemination (IUI) in infertile women.*
Conclusion: *Oocyte size and FSH concentration affect the outcomes of IUI; therefore, these conditions should be addressed before initiating treatment with assisted reproductive techniques.*

INTRODUCTION:

One of the indicators of infertility is the inability to achieve pregnancy after at least one year of regular, unprotected sexual intercourse, which may affect either the female or male reproductive system [1]. The World Health Organization indicates that approximately 17–20% of couples experience difficulty conceiving after one year of regular unprotected intercourse [2]. Assisted reproductive technologies, including intrauterine insemination (IUI), have been showing clear and continuous development, which improves the response of infertile couples to this technique. At the same time, there are physiological factors that require treatment, including maternal- and paternal-related causes [3]. Intrauterine insemination (IUI) is considered effective and safer in terms of clinical practice and cost compared to other techniques [4] Spermatogenesis is a complex process that may be disrupted by various factors, and there is a direct relationship between sperm DNA damage and male infertility [5]. The sperm DNA fragmentation index (DFI) reflects sperm integrity and is considered one of the key indicators for assessing semen quality [6]. Infertility is a global condition that affects millions of couples of reproductive age, and intrauterine insemination (IUI) is a crucial step in selecting high-quality sperm for fertilization, thereby increasing the chance of achieving pregnancy [7]. Reduced sperm quality is one of the main features that decrease the likelihood of fertilization and embryo formation, which later develops into the blastocyst stage [8]. Men are responsible for approximately 30% of infertility cases [9]. Semen analysis is regarded as the gold standard for assessing male fertility and is useful for monitoring spermatogenesis either before or after treatment [10].

Female-related factors also influence IUI outcomes, including age, the number of follicles, the size of the dominant follicle before stimulation, causes of infertility, and endometrial thickness [11]. There is no general consensus in previous studies regarding the optimal size of the dominant follicle on the day of human chorionic gonadotropin (HCG) administration; however, the hormone is usually given when the dominant follicle reaches 17 mm or more [12].

The thickness of the functional layer of the endometrium is not constant, as it continuously changes throughout the menstrual cycle. Once it reaches the required thickness for implantation, the fertilized oocyte embeds itself within the functional endometrial cells. If the endometrial thickness is insufficient for implantation, the embryo fails to implant and is shed [13].

The main causes of reduced endometrial thickness include poor blood circulation, inadequate growth rate of the endometrium, low estrogen concentration, progesterone dysfunction, improper use of certain medications, and persistent high levels of stress and anxiety [14]. Elevated levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary gland affect ovulation, the cervical mucus layer, and the blood vessels of the endometrium [15].

The concentration level of AMH does not affect the response to intrauterine insemination (IUI) in women younger than 35 years; however, this hormone had an effect in older women. This may be because younger patients not only have a better ovarian response but also better oocyte quality [16]. Elevated late follicular progesterone levels are associated with a reduced live birth rate following assisted reproductive technologies, which may be due to the premature advancement of endometrial changes, leading to a lack of synchronization between the embryo, implantation, and endometrial receptivity [17]. Identifying variables related to IUI outcomes and calculating prediction scores may help improve accurate counseling and optimize pre-treatment planning [18].

Objective of the present study To evaluate the effect of paternal factors such as the sperm DNA fragmentation index, maternal factors including hormone concentrations and follicular parameters, as well as menstrual cycle-related factors such as ovulation induction, timing, and procedures, on the effectiveness of IUI outcomes. The aim is to draw conclusions and provide objective counseling to infertile couples in accordance with their specific causes of infertility.

PATIENTS AND METHODS:

1. Patients:-

The present study included 96 infertile couples who attended the Ajeal Specialized Infertility Center in Kirkuk, Iraq, from January 2023 to the end of February 2025, seeking to achieve a response to intrauterine insemination (IUI) and obtain pregnancy. After completing the IUI procedures, the patients were divided into two groups: the first group included couples who responded to IUI and achieved pregnancy, while the second group included couples who did not respond to IUI and did not achieve pregnancy.

2- Inclusion Criteria:

The inclusion criteria for the couples were as follows: the male partner had to have normal semen according to World Health Organization (WHO) standards, and the female partners had to be aged between 20–40 years, with a normal uterus, patent fallopian tubes, and healthy ovaries free from advanced-grade cysts. These factors were confirmed using transvaginal ultrasound imaging. Male partners were additionally confirmed to meet WHO semen analysis criteria. Couples (both male and female) who did not meet the study criteria were excluded.

Procedure:

Couples were selected according to the current study criteria. Female patients were scheduled for assessment on the second day of their menstrual cycle for hormonal testing, including FSH, LH, progesterone, and AMH. Ovarian stimulation was performed by administering Letrozole 2.5 mg (Novartis, Switzerland) twice daily for five consecutive days. Patients were then monitored on days 11 and 13 of the menstrual cycle using ultrasound to identify mature follicles, in order to time the administration of human chorionic gonadotropin (HCG) (Livzon Farm, China, 1000 IU). Male partners were instructed to abstain from ejaculation for 3–5 days prior to semen collection. Semen was collected 24–36 hours after HCG administration, prepared, and washed using the Density-Gradient sperm separation method (SpermGrad, Sweden) before intrauterine insemination (IUI) was performed.

Intrauterine Insemination (IUI) Procedure:

After semen preparation using the previously described method, intrauterine insemination was performed using a sterile catheter connected to an insemination syringe, allowing the semen to be delivered into the uterine cavity. A volume of 0.8 mL of prepared semen was slowly injected after passing the catheter through the cervix. Prior to the procedure, it was ensured that the bladder was full, and the vulvar area was thoroughly cleaned using pre-prepared normal saline.

Assessment of Positive or Negative Outcomes of Intrauterine Insemination (IUI):

Fourteen to seventeen days after performing IUI, a beta-HCG test (Boditech Med Inc., Korea) was conducted to measure serum HCG levels in the participating female patients. A serum HCG concentration exceeding 25 IU/mL was considered indicative of a positive response to IUI, whereas a level below 25 IU/mL indicated a lack of response and was considered a negative result, with no pregnancy achieved. This represented the preliminary assessment. Confirmation of pregnancy (secondary assessment) was performed three weeks after the initial test using ultrasound to detect the presence of a gestational sac and placenta within the uterine lining.

Statistical Analysis:

Statistical analysis was performed using one-way analysis of variance (ANOVA) with letters A/B and the mean ± standard deviation (SD) to compare variable means between couples who responded and those who did not respond to intrauterine insemination (IUI). ANOVA was used to compare means across groups. Continuous variables were presented as mean ± SD, and comparisons between groups were performed using the T-test. Groups were stratified according to age categories for the IUI cycles. A p-value ≤ 0.05 was considered statistically significant. Potential confounding variables included female age, hormonal levels (FSH, LH, progesterone), and the number and size of ovarian follicles. For male partners, the sperm DNA fragmentation index (DFI) was assessed

RESULTS:

The study included 96 infertile couples aged 20–40 years. As shown in Table (1), there was a statistically significant increase in serum progesterone levels in infertile women who did not respond to intrauterine insemination (IUI) compared to responders across all age groups. Regarding LH levels, no significant differences were observed in the serum of IUI responders compared to non-responders across most age groups, except for the youngest age group (20–25 years), where a significant decrease in LH was observed in non-responders compared to responders. The mean LH level in responders of this age group was 6.070 ± 0.22 IU/L, whereas in non-responders it was 4.581 ± 0.216 IU/L.

Table (1) also demonstrated a significant increase in FSH levels in infertile female patients compared to non-responders across all age groups. For the 20–25 years age group, the mean FSH level in responders was 11.002 ± 0.481 IU/L, whereas in non-responders it was 8.519 ± 0.258 IU/L. In the 26–30 years age group, mean FSH levels in responders were 12.560 ± 0.259 IU/L compared to 8.856 ± 0.424 IU/L in non-responders. In the 31–35 years age group, mean FSH levels in responders were 11.765 ± 0.837 IU/L, whereas in non-responders they were 9.067 ± 0.497 IU/L. For the oldest age group (36–40 years), mean FSH levels in responders were 12.760 ± 0.00 IU/L, while in non-responders the mean level was 8.693 ± 0.447 IU/L.

Table (1) illustrates the relationship between IUI success and the effects of FSH, LH, and progesterone according to different age groups

(Progesterone **ng/ml**)	**LH mIU/ml**	**FSH mIU/ml**	**Age group**
0.715±0.014 A	6.070±0.227 A	11.002±0.481 A	**25-20 سنة Positive**
0.851±0.013 B	4.581±0.216 B	8.519±0.258 B	**25-20سنة Negative**
0.750±0.023	6.770±0.196	12.560±0.259	**30-26 سنة**

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A	A	A	Positive
0.865±0.016 B	5.522±0.312 A	8.856±0.424 B	30-26سنة Negative
0.707±0.023 A	5.655±0.268 A	11.765±0.837 A	35-31سنة Positive
0.825±0.026 B	5.819±0.298 A	9.067±0.497 B	35-31سنة Negative
0.710±0.00 A	5.12±0.00 A	12.760±0.00 A	40-36سنة Positive
0.856±0.021 B	5.36±1.099 A	8.693±0.447 B	40-36سنة Negative

The results presented in Table (2) showed a significant decrease in serum AMH levels in infertile women who did not respond to intrauterine insemination (IUI) compared to those who did respond, across all age groups. For the 20–25 years age group, the mean AMH level in responders was 3.760 ± 0.051 ng/mL, whereas in non-responders it was 2.705 ± 0.175 ng/mL. In the 26–30 years age group, mean AMH levels in responders were 3.560 ± 0.028 ng/mL compared to 2.246 ± 0.202 ng/mL in non-responders. For the 31–35 years age group, mean AMH levels in responders were 3.920 ± 0.115 ng/mL, whereas in non-responders they were 2.209 ± 0.222 ng/mL. In the oldest age group (36–40 years), the mean AMH level in responders was 3.490 ± 0.00 ng/mL, while in non-responders it was 2.158 ± 0.177 ng/mL.

Table (2): Shows the effects of DFI, AMH, number, size, and duration on IUI outcomes across different age groups.

AMH ng/ml	Largest egg size (mm)	Number of eggs Count	DFI (%)	Age group
3.760±0.051 A	21.00±0.267 A	2.500±0.327 A	86.750±0.977 A	25-20 سنة Positive
2.705±0.175 B	18.46±0.454 B	2.384±0.167 A	82.923±1.229 A	25-20سنة Negative
3.560±0.028 A	21.00±0.577 A	2.500±0.288 A	84.00±2.598 A	30-26 سنة Positive
2.246±0.202 B	18.50±0.312 B	2.400±0.210 A	83.50±1.497 A	30-26سنة Negative
3.920±0.115 A	22.00±1.154 A	2.00±0.577 A	87.500±0.288 A	35-31سنة Positive
2.209±0.222	18.30±0.291 B	1.700±0.146 A	82.500±2.122 A	35-31سنة Negative
3.490±0.00 A	18.46±0.00 A	2.500±0.00 A	85.00±0.00 A	40-36سنة Positive
2.158±0.177 B	18.33±0.512 A	2.00±0.288 A	76.667±2.890 B	40-36سنة Negative

The results in Table (2) also showed no significant differences in the number of mature oocytes on the day of insemination when comparing infertile women who responded to intrauterine insemination (IUI) with non-responders across all age groups.

Regarding follicle size on the day of insemination, Table (2) demonstrated a significant decrease in infertile women who did not respond to IUI compared to responders across all age groups, except for the oldest age group (36–40 years), where no significant difference was observed between responders and non-responders.

The results in Table (2) also indicated no significant differences in sperm DNA quality when comparing male partners of couples who responded to IUI with non-responders across all age groups, except for the oldest age group. In this group, a significant increase in sperm DNA fragmentation was observed in non-responders compared to responders. The mean level of normal sperm DNA in infertile male partners who responded to IUI was 85.00 ± 0.00%, whereas in non-responders it was 76.667 ± 2.890%.

DISCUSSION:

The outcomes of intrauterine insemination (IUI) are influenced by multiple factors, including treatment protocols, ovarian stimulation, insemination procedures, and semen preparation, as well as male factors, primarily sperm quality and related parameters such as sperm DNA fragmentation (DFI). Maternal factors, including hormonal levels, also play a significant role. This study focused on several maternal variables, including AMH, FSH, LH, progesterone, and the number and size of mature ovarian follicles on the day of insemination. Our study aimed to investigate the relationship between these variables and IUI response rates according to the study protocol, with patients stratified into four age groups.

The results demonstrated that positive IUI outcomes decreased with higher serum progesterone levels in infertile women at the early follicular phase. This finding is consistent with previous studies showing that clinical pregnancy rates following IUI decline with elevated serum progesterone levels [1]. The reduced pregnancy rates may be due to early endometrial maturation interfering with optimal timing for insemination during the follicular phase [19].

The study also showed that positive IUI outcomes decreased with lower serum AMH levels at the start of the follicular phase. This aligns with previous studies indicating that IUI response is negatively affected by reduced AMH levels [20]. Low AMH, secreted by granulosa cells of ovarian follicles, reflects a lower number of available follicles and consequently fewer mature oocytes, sometimes only one or none, which explains poor response to IUI [21].

FSH levels were also lower in non-responders compared to responders. This result is consistent with previous studies, which suggested that low FSH at the beginning of the follicular phase negatively affects IUI outcomes [22]. This may result from hypothalamic-pituitary axis insufficiency or dysregulated GnRH secretion from the hypothalamus, leading to impaired ovulation. Therefore, correcting low FSH levels prior to starting the IUI protocol is recommended [23].

In line with multiple studies, LH levels at the early follicular phase were not found to affect clinical pregnancy outcomes following IUI [24]. This is likely because LH is not directly involved in follicular growth [25-29].

CONCLUSION:

IUI response rates decrease with increased sperm DNA fragmentation and with reduced FSH levels and follicle size. In contrast, LH levels and the number of mature oocytes on the second day of the menstrual cycle do not significantly affect IUI outcomes.

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