OPEN ACCESS

Middle East Research Journal of Medical Sciences
ISSN: 2789-7699 (Print) & ISSN: 2958-2024 (Online)
Frequency: Bi-Monthly
DOI: 10.36348/merjms.2024.v04i03.004

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Factors Affecting Pregnancy and Live Birth Rates in Gonadotropin Treated Intrauterine Insemination Cycles of Unexplained Infertile Patients

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Abstract: ***Objective*:** We studied how basal hormone levels, the type of gonadotropin used for ovarian stimulation, initial gonadotropin dose, total gonadotropin dose, cycle duration, follicle size on the day of (human chorionic gonadotropin) hCG administration, and endometrial thickness affect pregnancy success in unexplained infertile patients undergoing IUI cycles. ***Materials and Methods:*** A total of 299 patients aged 20-40 years with unexplained infertility underwent ovulation induction (OI) and IUI treatment with gonadotropins were included in the study. Clinical pregnancy and live birth were primary outcomes. ***Results:*** Out of 299 patients, 11% (n=36) achieved clinical pregnancies, 9.9% (n=29) resulted in live births, and 2.3% (n=7) ended in abortions. Comparing age, basal FSH, LH, and E2 values between groups, it was found that E2 levels in the live birth group were significantly lower than those in the no-pregnancy group (39 pg/mL vs. 46.95 pg/mL, p=0.029). However, there were no significant differences in initial gonadotropin doses and total gonadotropin doses between the two groups (p=0.749, p=0.113). Patients in the no-conception group had significantly shorter cycle durations (mean=8.71 days) compared to patients in the live birth group (mean=9.55 days) (p=0.017). Endometrial thickness did not differ significantly between the groups (p=0.542). Follicle size in the no-conception group (mean=17.22 mm) was significantly smaller than in the live birth group (mean=19.11 mm) (p=0.176). There was no statistically significant difference between the rFSH or HMG groups for ovarian stimulation (p=0.487). ***Conclusion***: IUI is an acceptable treatment method with a 9.69% live birth rate. In our study, basal E2 level of 39 pg/mL, cycle duration of 9.5 days, and 17 mm follicle size were found to be associated with live births.

Keywords: İntrauterine insemination, live births, ovarian stimulation, unexplained infertility.

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Research Paper

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How to cite this paper:
Latife Nevin Pehlivanlar Dar *et al* (2024). Factors Affecting Pregnancy and Live Birth Rates in Gonadotropin Treated Intrauterine Insemination Cycles of Unexplained Infertile Patients. *Middle East Res J. Med. Sci*, *4*(3): 61-65.

Article History:
| Submit: 09.05.2024 |
| Accepted: 10.06.2024 |
| Published: 15.06.2024 |

INTRODUCTION

Infertility is defined as the failure to achieve pregnancy despite adequate and regular intercourse without the use of any contraceptive method for one year in a couple under 35 years of age, and within six months for a couple over 35 years of age [1]. Approximately 10% of couples worldwide suffer from infertility [2]. Despite advancements in diagnostic methods, the cause of infertility remains unexplained in 25% of infertile couples. Unexplained infertility is a diagnosis of exclusion, requiring normal sperm analysis, hysterosalpingography (HSG), and ovarian reserve testing [3, 4].

Assisted reproductive technologies (ART) are widely used and effective treatment methods for unexplained infertility. Intrauterine insemination (IUI) is considered the first-line treatment for unexplained infertility due to its effectiveness, affordability, and non-invasive nature [5].

We retrospectively analyzed the cases of achieved and unachieved pregnancies obtained through the IUI technique in women (aged 20-40) with unexplained infertility who presented to our clinic between 2018 and 2020. In our study, we aimed to investigate the relationship between the type of gonadotropin used, cycle length, endometrial thickness on the day of hCG administration, and follicle size with respect to pregnancy in unexplained infertile patients. Since there is no correctable organic cause in unexplained infertile patients, we think that studies

presenting the ovulation induction cycle parameters in which pregnancy is high are valuable.

MATERIALS AND METHODS

A total of 299 female patients (age range: 20-40 years) experiencing infertility for at least 1 year, with tubal patency confirmed by HSG, and diagnosed with unexplained infertility, whose partners had normal sperm count, motility, and morphology parameters according to the World Health Organization (WHO) 2010 criteria, were included in the study. Demographic data such as age and basal hormone levels of the patients were recorded. Patient information was provided retrospectively from medical records. The hospital's institutional ethical committee granted approval for the study and exempted the need for informed consent from the participants, citing the retrospective nature of the study.

Patients with concomitant systemic diseases, body mass index (BMI) over 30 kg/m², and those with known causes of infertility (such as endometrioma/endometriosis, polycystic ovarian disease, hypogonadotropic hypogonadism, male subfertility) were excluded from the study.

Initial infertility investigations (basal hormone levels, semen analysis, complete gynecological examination) were conducted for all couples. Tubal patency was confirmed by HSG. Transvaginal ultrasound (TV-USG) performed on the 2nd or 3rd day of the menstrual cycle ruled out persistent cysts, and individualized doses of gonadotropins (Gonal F, Serono, Basel, Switzerland – Meriofert, IBSA, Lamone, Switzerland) were initiated according to BMI. Regular TV-USG examinations were performed until the dominant follicle was observed. When the dominant follicle reached a size >17mm, hCG (Ovitrelle, Merck, Modugno, Italy) was administered as a trigger. On the day of hCG trigger, endometrial thickness and follicle sizes of the patients were measured using Mindray DC-60 TV-USG. Semen analyses were evaluated according to WHO 2010 standards (sperm count >20x106 /ml, total sperm count >40x106 /ml, progressive motility >50%, normal morphology >30% according to WHO criteria, >14% according to Kruger Strict criteria). Patients were advised to observe 3-4 days of sexual abstinence before semen collection, and samples were obtained via masturbation prior to IUI. Following a 30-minute liquefaction period at 37°C, semen was centrifuged with Earl's balanced salt solution at ratios of ½ and 1/1. After centrifugation, the pellet was mixed with 1cc Universal Medium, incubated at 35°C, and the most active spermatozoa were selected using the swim-up technique for insemination. IUI was performed using a Gynetics brand insemination catheter inserted through the cervix 36 hours after hCG injection. All patients were provided with luteal support using intravaginal Progesterone (P) 200 mg once daily (Progestan, Koçak Farma, Üsküdar, Istanbul), and were scheduled for beta hCG testing 12 days later. Patients with confirmed pregnancies were followed up, and those with fetal cardiac activity were considered clinically pregnant, while births at or beyond 24 weeks of gestation or weighing over 500 grams were considered live births.

Statistical Analysis

SPSS 25 software was used for statistical analysis. Descriptive statistics were used to present demographic data of the patients. Due to the unequal distribution of patients among the pregnancy groups, non-parametric tests were used for analysis. The Kruskal-Wallis test, a non-parametric alternative to one-way analysis of variance (ANOVA), was used to determine the relationship between age, hormonal values follicule stimulating hormone, luteinizing hormone, estradiol (FSH, LH, E2), gonadotropin, follicle size, endometrial thickness, total drug dose, cycle duration, and pregnancy outcome. In cases where significant results were obtained, the Mann-Whitney U test, a non-parametric alternative to the independent samples t-test, was used to determine differences between groups.

DISCUSSION

The International Committee for Monitoring Assisted Reproductive Technologies (ICMART) defines unexplained infertility as a condition in couples who have normal ovarian function, fallopian tubes, uterus, cervix, and pelvis, with adequate sexual intercourse frequency, and whose partners have normal testicular function, genitourinary anatomy, and normal ejaculate [6]. Studies providing ideal treatment and cycle parameters that can increase pregnancy rates are valuable for the literature since in unexplained infertility, there is no organic cause that can be corrected to achieve pregnancy. In our study, we aimed to present cycle parameters induced by gonadotropins in IUI cycles that would increase pregnancy and live birth rates in this specific patient group.

An increase in estrogen reduces uterine blood flow and hinders implantation and trophoblast invasion [7, 8]. It has even been associated with intrauterine growth restriction (IUGR) and hypertensive disorders [9, 10]. Studies have shown that E2 levels below 80 pg/mL have a positive effect on pregnancy [11]. Garcia *et al.*, found that in IUI cycles of 93 patients, pregnancy was not achieved with estradiol levels below 21 pg/mL or above 87 pg/mL [12]. In our study, the average estradiol level in the group with live births was 39.1 pg/mL, while in the group unable to conceive, it was 46.7 pg/mL. Although both estrogen values were within normal

limits, the lower estrogen level in the group with live births was statistically significant (p=0.29). This suggests that increased estrogen may hinder implantation by reducing uterine blood flow.

The combination of OS with IUI is a commonly used treatment method in infertile patients, and there are many studies in the literature regarding the success of different stimulation agents. In a study by Koçak *et al*., involving 50 patients, the total dose of recombinant follicle-stimulating hormone (rFSH) was 710 IU, while in the group receiving human menopausal gonadotropin (HMG), the total dose was 636 IU. The mean cycle duration was 9 days in the rFSH group and 8.3 days in the HMG group. This study found no significant difference in total doses or cycle durations between different agents [13].

However, in a retrospective study conducted by Li *et al*., comprising 361 patients and a total of 930 IUI cycles, they found that clinical pregnancy was associated with high gonadotropin doses (>750 IU) (p<0.05) [14]. In our study, the total gonadotropin dose was 816.38 ± 389.78 IU (p=0.749), and no significant difference was found regarding the effect of total medication dose on pregnancy. However, when examining stimulation durations, our study found a significant statistical difference between the groups that did and did not achieve pregnancy. The mean cycle duration was 9.5 days in those who achieved pregnancy, while it was shorter at 8.7 days in the group that did not achieve pregnancy (p=0.017).

During the menstrual cycle, the endometrium undergoes cyclical changes in preparation for implantation. During the follicular phase, growing follicles produce increasing amounts of E2, inducing proliferative endometrial changes. After ovulation, the corpus luteum produces P, leading to secretory changes. Since serum hormonal levels of E2 and P may not always accurately predict endometrial development and other methods like histological studies are highly invasive, ultrasonography has been used as a non-invasive technique for monitoring infertile women for over two decades [15]. In a retrospective study examining 1036 IUI cycles conducted by Yiwen Liu *et al*., the minimum endometrial thickness for achieving pregnancy was found to be 5 mm, with a maximum thickness of 20 mm. They found that pregnancy did not occur below 5 mm or above 15 mm, and the ideal endometrial thickness for achieving pregnancy was between 10.5 and 13.9 mm [16]. Similarly, in a study published in 2023, comprising 1232 IUI cycles, Huang *et al*., found that clinical pregnancies were highest with endometrial thickness ranging from 8 to 12 mm [17]. In our study, the mean endometrial thickness on the day of hCG administration was 10 mm in the group that achieved pregnancy (29 patients), while it was 15 mm in the group that did not achieve pregnancy (263 patients). Although these endometrial thicknesses differed numerically, they were not statistically significant (p=0.542).

The success of insemination depends on various factors, one of which is the optimal follicle size on the day of hCG administration. Studies have mentioned that hCG administration at a lead follicle size of 21.1-22.0 mm is associated with higher odds of clinical pregnancy [18]. In a study by Maher *et al*., analyzing 512 cycles of OS with gonadotropins, the optimal follicle size for increasing pregnancy rates was found to be 19-20 mm [19].

Pekcan *et al*., involved 352 IUI patients, in their study. In this study, the mean follicle size was 19 mm for patients who achieved live births and 19.1 mm for those who did not achieve pregnancy [20]. In our study, the mean follicle size after IUI was 19.1 mm in the group that achieved pregnancy (29 patients) and 17.2 mm in the group that did not achieve pregnancy, showing a statistically significant difference (p=0.041).

OS medications increase the number of follicles in the ovaries, thereby increasing cycle fecundity. There are numerous studies investigating the effect of the type of gonadotropin used on pregnancy. In our retrospective study, the total number of patients who achieved pregnancy was 29, with 18 (62.1%) undergoing stimulation with rFSH and 11 (37.9%) with HMG, resulting in live births (p=0.487). However, there was no statistically significant difference between the type of gonadotropin used and pregnancy rates. A meta-analysis conducted by Wely *et al*., in 2003 found no superiority between rFSH and HMG [21]. A retrospective study by Demirol *et al*., in 2006 concluded that rFSH was superior to HMG [22]. In a study by Kocak *et al*., involving 50 participants, two pregnancies were achieved in each group, and no significant difference was found between rFSH and HMG [13]. In another study by Cicek *et al*., 36 patients were treated with rFSH and 23 with HMG. The live birth rate was 17% in the rFSH group and 8% in the HMG group, with no statistically significant difference [23].

Although IUI is a cheap, easily applicable, and non-invasive method with an acceptable pregnancy rate, the NICE guidelines recommend IVF for unexplained infertility lasting more than 2 years [24]. The limitation of our study was the lack of recorded infertility durations in the files, hence we could not make any recommendations regarding infertility duration and clinical pregnancy and live birth.

RESULTS

A total of 299 patients who underwent IUI for unexplained infertility were retrospectively screened, excluding patients with cycle cancellations for various reasons.

Out of the total 299 patients who underwent IUI, 7 pregnancies (2.3%) ended in miscarriage, while 29 (9.69%) resulted in live births and total number of clinical pregnancies obtained was 36 (11.9%). When comparing demographic characteristics and basal FSH, LH, and E2 values between the group that achieved pregnancy and the group that did not, no significant statistical differences were found except for E2. The E2 levels in the group that did not achieve pregnancy had a mean of 46.95 pg/mL, compared to 39 pg/mL in the group that achieved pregnancy (p=0.029). There were no significant statistical differences between the two groups in terms of starting gonadotropin doses and total gonadotropin doses (p=0.749, p=0.113). The cycle durations of patients in the group that did not achieve pregnancy (mean=8.71 days) were significantly lower than those in the group that resulted in live births (mean=9.55 days) (p=0.017). There was no significant statistical difference between endometrial thickness and pregnancy outcomes (p=0.542). The results showed statistically significant differences in the mean follicle size measured on the day of hCG administration. The mean follicle size of patients in the group that did not achieve pregnancy (mean=17.22 mm) was significantly lower than that of patients in the group that resulted in live births (mean=19.11 mm) (p=0.176). When evaluating pregnancy outcomes in patients treated with rFSH or HMG for OS, no statistically significant difference was found (p=0.487). The descriptive statistics of the patients are shown in Table 1, and the clinical and laboratory characteristics of patients who achieved pregnancy and those who did not are shown in Table 2.

Table 1: Descriptive Statistics of Patients Regarding Variables Used in the Study

	n/avg & (Ss)
Age	28.38 ± 4.33
FSH(mIU/mL)	6.67 ± 2.18
LH(mIU/mL)	5.55 ± 2.58
E2(pg/ml)	43.85 ± 16.98
Cycle Duration(day)	8.81 ± 2.29
Gonadotropin Dose(IU)	81.65 ± 25.99
Total Drug Dose (IU)	776.31 ± 438.13
Endometrial thickness(mm)	14.44 ± 75.40
Follicle size(mm)	17.37 ± 2.84

Table 2: Relationship between Age and Hormonal Variables, Cycle Duration, Gonadotropin Dose, Total Drug Dose, Endometrial Thickness, Follicle Size and Pregnancy Outcomes

	Live Birth (n=29)	**Miscarriage (n=7)**	**Not Pregnant (n=263)**	**P Value**
FSH	6.90 ± 2.28	7.28 ± 2.09	7.07 ± 4. 18	.608*
LH	6.90 ± 2.28	5.81 ± 3.59	5.53 ± 2.56	.749*
E2	39.19 ± 17.05	41.60 ± 21.33	46.75 ± 19.58	.029*
Endometrial Thickness (mm)	10.04 ± 1.41	9.26 ± 1.44	15.07 ± 80.41	.542*
Follicle Size(mm)	19.11 ± 1.69	18.33 ± 1.15	17.20 ± 2.90	.041*
Cycle Duration (days)	9.55 ± 1.64	9.57 ± 1.51	8.71 ± 2.35	.017*
Gonadotropin Dose (IU)	82.76 ± 23.24	75.00 ± 0.00	81.70 ± 26.64	.749*
Total Drug Dose (IU)	816.38 ± 389.78	835.71 ± 257.74	770.19 ± 447. 69	.113*
rfSH	18 ± 62.1	11 ± 37.9	169 ± 64.8	.487*
HMG	11 ± 37.9	1 ± 14.3	83 ± 31.4	
Combined	0 ± 0	0 ± 0	10 ± 3.8	

CONCLUSION

IUI is a preferable method in patients with a short appropriate infertility duration, with acceptable live birth rates. In our study, basal E2 levels of 39 pg/mL, a cycle duration of 9.5 days, and a follicle size of 19 mm were found to be significant for live births.

Acknowledgments: Not applicable.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.
Ethics Statement: This study was approved by the local ethical committee. NO: 276708

Funding: The authors received no financial support for the research, authorship, and/or publication of this article.

Supplementary Material: None

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