

## Comparing the Effectiveness of Doing Intra-uterine Insemination 36 and 42 Hours After Human Chorionic Gonadotropin (HCG) Injection on Pregnancy Rate: A Randomized Clinical Trial

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### Abstract

**Objective:** Intrauterine insemination (IUI) is an assisted conception technique that involves the deposition of a processed semen sample in the upper uterine cavity, overcoming natural barriers to sperm ascent in the female reproductive tract. Hence, we compared the results of doing intra-uterine insemination 36 and 42 hours after human chorionic gonadotropin (hCG) hormone injection to achieve clinical and chemical pregnancy rates.

**Materials and methods:** One hundred and sixty infertile women with unexplained infertility participated in this clinical trial. They were divided into two groups: those who underwent IUI 36 hours after hCG injection (control group), and those who underwent IUI 42 hours after hCG injection (case group). Statistical analyses were done using IBM-SPSS 25.0. and Chi-square test were used for data analysis.

**Results:** The percentages of clinical and chemical pregnancies were significantly higher in the 42h group compared to the other group ( $P = 0.038$  vs.  $P = 0.009$ , respectively). There was no significant difference regarding frequency of abortion, twin and ectopic pregnancies between the two groups ( $P > 0.05$ ).

**Conclusion:** Doing IUI 42 hours after hCG injection can significantly increase chances of fertility compared to doing it 36 hours after hCG injection.

**Keywords:** Intra-Uterine Insemination; Infertility; Human Chorionic Gonadotropin; Injection Time

### Introduction

According to the World Health Organization (WHO), infertility is defined as a reproductive system disease

in which failure to achieve a pregnancy after 12 months or more of regular unprotected sex fails (1). There are many reasons for infertility that medical intervention can treat in some cases (2), such as expectant treatment (lifestyle change and timely intercourse), ovarian stimulation with clomiphene citrate (CC), gonadotropins or combination with CC.

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Ovarian stimulation is followed by intrauterine insemination (IUI), in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) (3).

Intra-uterine insemination (IUI) with or without ovarian stimulation is an assisted reproductive technique. It can be the first treatment choice for unexplained infertility, infertilities because of mild male factor, minimal and mild endometriosis, mild tubal factor, anovulation, and ejaculatory disorders (4). It is a simple, noninvasive and cost-efficient procedure compared to the other methods. The requirements for doing intra-uterine insemination are occurrence of ovulation in the IUI cycle, patency of at least one fallopian tube, insemination with a sufficient number of motile sperms, and not having any cervical or uterine anomaly or pelvic infections (5-7). IUI with controlled ovarian stimulation is a suitable, non-invasive and low-cost treatment for unexplained infertility that is done before in vitro fertilization. It has been suggested that doing IUI with controlled ovarian stimulation is more effective than doing IUI without it (7, 8).

Clinical pregnancy rates have been 11% to 33% after doing IUI for all infertility causes (9). There are many indicators for its success rate, including age, infertility period, ovarian stimulation protocol, infertility etiology, numbers of treatment cycles and pre-ovulatory follicles at the time of human chorionic gonadotropin (hCG) injection, timing of IUI, total motile sperm count more than 10 million and insemination count more than one million with more than 4% normal sperm morphology (4, 8, 10).

The ovulation time and its interval until doing IUI can be influential on the IUI outcome. Natural ovulation usually occurs 37 to 39 hours after the start of luteinizing hormone surge (11). Follicular rupture and ovulation usually occur 36 to 38 hours after hCG injection (12). The optimal timing of IUI for improving pregnancy outcome has been mentioned 12, 18, 36-40 hours after hCG injection (13).

Different studies have investigated the optimum interval from hCG injection to IUI and also double IUI in an ovulation induction cycle (14-17). Still, there is no agreement on its optimal time. In most studies, intra-uterine insemination is done 32 or 36 hours after hCG injection. Also, a systematic review that compared this issue 24 and 48 hours after hCG injection did not find any significant difference in pregnancy rates (18). The results of Soliman's research showed a significant increase in pregnancy rates in women with delayed IUI 48 h after hCG injection, compared with controls (19).

Yumusak and colleagues reported that fertility potential in infertile women is increased by IUI performed at either 24 hours or 36 hours after ovulation triggered by hCG injection (5). This study compared the chemical and clinical pregnancy rates of doing IUI 36 and 42 hours after hCG injection in women with unexplained infertility.

## Materials and methods

**Study design:** This study was conducted within Ali Ibn Abi Talib which is a referral educational hospital and a tertiary center of Sistan and Balouchestan province in Zahedan, Iran. One hundred and sixty infertile women with unexplained infertility participated in this clinical trial. Six of them were excluded according to exclusion criteria (Figure 1). The rest were randomly divided into two groups (n = 77 in each group): 1) those who underwent IUI 36 hours after hCG injection (control group), and 2) those who underwent IUI 42 hours after hCG injection (case group). All participants signed an informed consent before entering the study. The study protocol was approved by the ethics committee of our university (IR.ZAUMS.REC.1396.347).

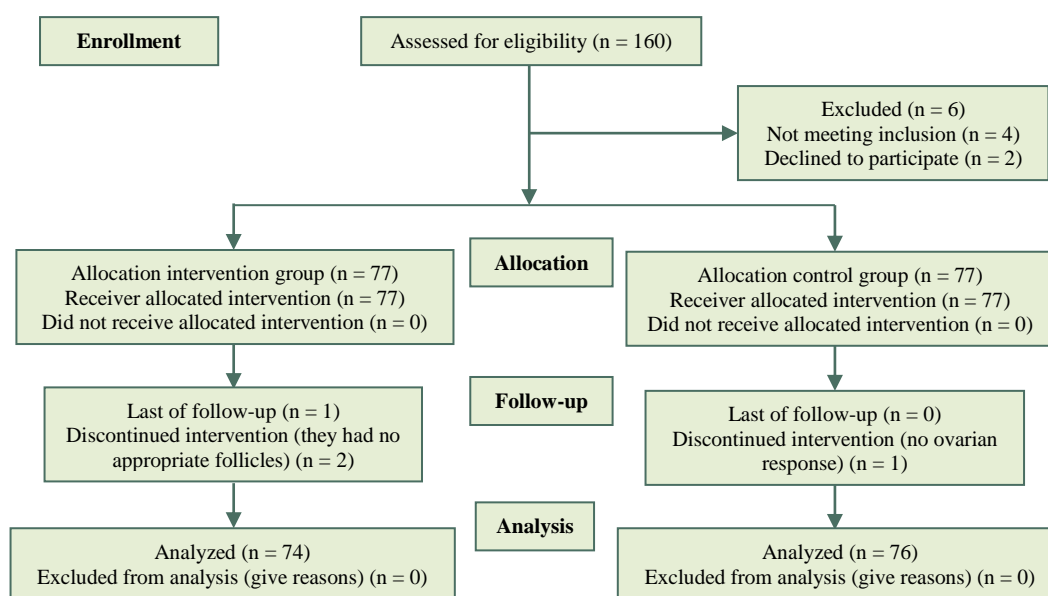
**The inclusion and exclusion criteria:** The inclusion criteria were: 1) having unexplained infertility, 2) being infertile for at least one year after trying to become pregnant, and 3) undergoing IUI for the first time.

The exclusion criteria were having: 1) endocrine disorders such as thyroid or diabetes mellitus, 2) history of IUI, 3) presence of four or more dominant follicles in the ultrasound imaging before hCG injection, and 4) not having at least two follicles  $\geq 18$  mm on the days of hCG injection.

**Study Description:** All participants were revisited once in the first three days of the menstrual cycle. Transvaginal ultrasound imaging was done to evaluate the absence of ovarian cyst. We administered Letrozole (Letrozole, tablet, 2.5 mg, LETROZOLE-SOHA -IRAN2/5) to all of them from the third day, 5 milligrams per day for five days. Then, on the 10<sup>th</sup> day of the menstrual cycle, we did another transvaginal ultrasound imaging for all participants. If needed, we administered one or two HMG injections (menotropins Daroupakhsh 75IU AMP-HUMEGNAN). They were re-examined for follicles under transvaginal ultrasound imaging a few days later.

If there were one or two follicle(s)  $\geq 18$  mm in the ultrasound imaging, we injected 10,000 units of hCG (HCG D.P (FOLIGNAN) 5000U VIAL-Daroupakhsh).

## Time Interval for IUI after HCG Injection



**Figure 1:** Consort flowchart of the study

We did IUI 36 hours after hCG injection in one group (control group) and 42 hours after hCG injection in the other group (case group). IUI was done the same way for all participants.

The semen was contained in a sterile container after two days of abstinence until one or two hours before IUI. It was prepared for use by swim up double wash technique with Hamz F10 culture media. We prepared 0.5 cc of semen for each participant by a special catheter for transferring sperm (catheter cook medical) and it was injected with an insulin syringe. The injection was done slowly into the cervix. Afterwards, the participants were placed in supine position for 15 minutes. All participants were advised to have sexual intercourse when the follicle diameter became 16 mm and also 12 hours after IUI.

The day after IUI, all patients received 400 mg of progesterone (400 mg cyclogest suppository manufactured by ACTOGEST, producer ACTOVERCO). Sixteen days after hCG injection, serum  $\beta$ -hCG was measured to assess pregnancy. If it was positive, chemical pregnancy was considered. In such cases, transvaginal ultrasound imaging was done four weeks after the positive pregnancy test to assess clinical pregnancy and the presence of pregnancy sac. The pregnant patients were followed up for the occurrence of abortion, ectopic, twin and ongoing pregnancies until the end of the 12<sup>th</sup> week of gestation.

**Data collection:** All couples were evaluated to have a normal semen analysis, uterus and fallopian tubes with hysterosalpingography (or laparoscopy

and hysteroscopy) before the study. Transvaginal ultrasound imaging was done to examine the uterus, tubes and ovaries. Hormonal evaluation was done with assessing follicular stimulating, luteinizing and estradiol (E2) hormones on the third day of menstruation, as well as serum prolactin, thyroid-stimulating and anti-mullerian hormones.

The semen analysis and hormone test results were normal in all participants. Presence of serum progesterone in the mid-luteal phase was considered as a sign of ovulation. The cervical and uterine cavities were normal in the examination and transvaginal ultrasound imaging. At least existence of one tube was proven by hysterosalpingography or laparoscopy.

**Statistical Analysis:** The statistical package for social sciences (SPSS) software version 25 was used for the data analysis. Continuous variables were reported with mean  $\pm$  standard deviation and analyzed by the independent t-test. The values were compared with chi-square test. P value less than 0.05 was considered significant.

## Results

One hundred and sixty women with unexplained infertility participated in this clinical trial. Six participants were excluded from the study at the beginning because of having the exclusion criteria. Four other participants were excluded during the study (Figure 1). So, the data of 150 participants were analyzed. They were 18 to 35 years old and had a body mass index of 18 to 30 kg/m<sup>2</sup>. There was

no significant difference in means of age, body mass index and infertility duration between the two groups. Also, the average levels of prolactin, anti-mullerian hormone, thyroid-stimulating and follicular stimulating hormones were not significantly different between them (Table 1).

**Table 1:** Basic characteristic (mean  $\pm$  standard deviation)

	Control (36h) group	Case (42h) group	P value
Age (years old)	29 $\pm$ 4	29 $\pm$ 5	0.267
BMI (kg/m <sup>2</sup> )	24 $\pm$ 4	25 $\pm$ 5	0.164
Infertility duration (years)	5.1 $\pm$ 3.4	4.9 $\pm$ 3.3	0.668
AMH (ng/dl)	3.3 $\pm$ 2.7	4.3 $\pm$ 3.1	0.115
Prolactin (mIU/L)	111 $\pm$ 53	101 $\pm$ 48	0.774
TSH (mIU/L)	2.2 $\pm$ 1.3	2.5 $\pm$ 1.9	0.370

FSH: Follicular stimulating hormone

The chemical pregnancy rates were 32% in the case group and 14% in the control group. The difference was significant ( $P = 0.009$ ). The clinical pregnancy rates were 28% in case group and 14% in the control group which was significantly different ( $P = 0.038$ ). The frequency of abortion and twin pregnancies were not significantly different between the two groups (Table 2).

**Table 2:** Treatment outcomes

Patients	Control (36h) group (n = 76)	Case (42h) group (n = 74)	P value
Chemical pregnancy	11 (14%)	24 (32%)	0.009
Clinical pregnancy	11 (14%)	21 (28%)	0.038
Abortion	0	3 (4.1%)	0.076
Twin pregnancy	0	1 (1.4%)	0.309

## Discussion

Controlled ovarian hyper-stimulation and IUI have been used to treat sub-fertility caused by mild male factor, anovulation, mild endometriosis, and unexplained infertility. This method is popular among infertile couples because of its low costs (20-22). Important factors affecting outcome of controlled ovarian hyperstimulation-IUI include age, IUI symptoms, sperm preparation, insemination methods and the the timing of administration of IUI as the most factor among them (5).

In present study chemical pregnancy rates were 32% in the case group and 14% in the control group, which was significantly different. The pregnancy rate is higher when IUI is done 42 hours compared to 36 hours after hCG injection. Some studies indicate that

ovulation occurs 36 to 48 hours after hCG injection (23, 24). The efficacy of the IUI technique is due to ovarian stimulation caused by more than one follicle and the preparation of sperm in the uterus close to the time of possible ovulation (25). The oocyte is successfully fertilized when fertilization begins 6 hours after ovulation. This means that at the time of ovulation, even if the spermatozoa meet the oocyte, it still has 6 hours to start the ovulation period. This indicates that IUI after ovulation is better than IUI before or even IUI at ovulation time (19). Because in stimulated cycles, ovulation often occurs 36 hours after hCG injection, IUI is traditionally performed 34-36 hours after hCG injection, although different protocols have been reported from 12 to 60 h after hCG injection. The reason of this work is the optimal endometrial secretion that allows better implantation of fertile embryos. In addition, the rupture of follicle occurs between 36 and 48 hours after hCG injection and may increase the chance of a successful pregnancy because of a synchrony between ovulation and insemination (14, 19). The presence of a sufficient number of motile sperms in the fallopian tubes when ovulation occurs can increase the chance of pregnancy in the IUI cycles (26).

In present study the clinical pregnancy rates were 28% in case group and 14% in the control group which was significantly different. Although Elhddad and Saad reported a clinical lower pregnancy rate of 15.5% per cycle IUI (3).

Kamel and colleagues compared the results of IUI procedure 36 and 48 hours after hCG injection on 150 women with unexplained infertility. They found out that the number of pregnancies were significantly higher in the 48-hour group (7). This study was similar to our work and Soliman's study (19). Weiss and colleagues reported that percentages of clinical pregnancies in three groups (36, 42 and 48 hours after hCG injection) were 20%, 38% and 24% respectively. Although the percentage was higher in the 42-hour group, it was not significant. Also, there was no significant difference between the three groups regarding twin pregnancy (14). Their number of participants was less than ours (92 vs. 150 women). Still they had a high pregnancy rate when they did IUI 42 hours after hCG injection.

Ghanem and colleagues observed that 816 of 1114 participants had ovulation at the time of IUI (73%). They reported the reason for infertility in their participants was the mild male factor, anovulation, and unexplained infertility. They observed that when



ovulation has occurred, IUI results in higher clinical pregnancy rates compared to when ovulation has not happened. Of course, when male and non-male factor subgroups were examined, this difference was not observed in the male factor subgroup. Clinical pregnancy rate was significantly higher in the unexplained infertility group with ovulation occurrence (23, 24, 26).

Agrawal and colleagues investigated the difference between doing IUI between 36 to 40 hours and more than 40 hours after hCG injection. They assessed 624 cycles and concluded that the pregnancy rate is significantly higher when IUI is done between 36 to 40 hours after hCG injection (27). The problem of their work is that they have not indicated a precise time. More than 40 hours is too vast and 36 to 40 hours is not conclusive enough. Also, their participants were infertile because of many reasons and this can be a defective factor.

In a systematic review, Cantineau and colleagues evaluated the results of 18 randomized clinical trials. This included 2279 infertile women. They concluded that there seems to be no significant difference between doing IUI from 24 to 48 hours after hCG injection (18). Some other studies have mentioned the same conclusion (12, 17, 28).

Probably the difference in these results might be related to the studied population. In most cases women with different reasons of infertility were included in the study which can be a defective factor. Unlike most of the other studies, we included only women with unexplained infertility and excluded all the rest whose infertility reasons were known. All women were doing their first IUI cycle in our study. So, unlike some of the studies, women who had done IUI in the past were excluded from our work. This can reduce the bias between the two study groups.

There are diverse reported pregnancy rates after doing IUI from 11 % to 33.3% (9, 24). 28% clinical pregnancy in our study might be because of the higher chances of the studied population in becoming pregnant. It seems that women with unexplained infertility who undergo IUI for the first time have a higher chance of becoming pregnant compared to other infertile women.

Some of the limitations of our study were the number of participants and not evaluating the percentage of live births. Since this was based on a residency dissertation, we had a limited time to gather more participants and follow-up each case until birth.

Success in IUI depends on several factors, so there

is still debate about the factors affecting patient selection and the optimal protocol used to improve pregnancy rates after IUI (3, 29, 30). In the future the factors affecting the outcome of IUI can be examined.

## **Conclusion**

It seems that doing the IUI procedure 42 hours after hCG injection can significantly increase the clinical and chemical pregnancy rates of women with unexplained infertility. More studies are recommended on this subject, especially to find out the best precise time for doing IUI after hCG injection for having the highest success rate. Also, following up the participants until they give birth is recommended.

All participants signed a written informed consent before entering the study. The study protocol was approved by the ethics committee of our university (IR.ZAUMS.REC.1396.347).

## **Conflict of Interests**

Authors have no conflict of interests.

## **Acknowledgments**

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