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Hysterosalpingography prior to the gonadotropin stimulated intrauterine insemination improves clinical pregnancy rates in women with unexplained infertility

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Summary

Purpose of investigation: Gonadotropin stimulated intrauterine insemination (IUI) cycles performed following one month after hysterosalpingography (HSG) are associated with improvement in clinical pregnancy rates in unexplained infertile couples. *Materials and Methods:* A retrospective cohort study was performed between 2008 and 2014. A total of 92 unexplained infertile couples undergoing their first cycle IUI stimulated by gonadotropins were included in the analysis. Participants were classified into two groups according to IUI cycles performed one month (Group A, n = 25 cycles) or longer than one month (Group B, n = 67 cycles) after the HSG procedure. *Result:* The overall clinical pregnancy rate was found as 25% (23 clinical pregnancies / 92 cycles). Clinical pregnancy rate was 44 % (11/25) for Group A and 17.9 % (12/67) for Group B. In Group A, there were significantly higher clinical pregnancy rates compared to Group B (OR: 3.6, 95% CI, 1.3–9.8; $p = 0.012$). *Conclusions:* It has been demonstrated that fertility improving effect of HSG was most prominent in the first six months after procedure. Likewise, in gonadotropin stimulated IUI cycles performed following one month after HSG, there seems to be an improvement in pregnancy rates in unexplained couples. In unexplained cases, it may be a reasonable approach to plan IUI cycles in the first month after HSG in clinical practice.

Key Words: IUI; Ovarian stimulation; Infertility; Hysterosalpingography.

Introduction

Hysterosalpingography (HSG) is widely used for the female infertility workup [1]. Despite its moderate sensitivity, HSG continues to be a valuable test with a high specificity for evaluating tubal patency and uterine cavity abnormalities [1, 2]. Also, it is less expensive, less invasive, and easier to perform compared to laparoscopy which is the considered the best diagnostic procedure in tubal patency.

Many clinicians think that there would be an increase in the probability of a patient to become pregnant in the first few months after the HSG procedure. This idea was based on an evidence put forward 50 years ago which has shown that the conception rates were increased in the first four months after HSG procedure performed with an oil-soluble contrast media (OSCM) compared to those of same time before the procedure [3]. Since that, a number of studies have investigated the therapeutic effect of OSCM used during HSG and they have found that it was associated with increasing fertility rates for the first few months after the procedure especially in women having bilaterally normal tubal anatomy [1, 3-5]. As a result, "tubal flushing" term has taken place in the literature as a method for providing fertility, even though exact mechanism is not completely understood. However, all of the studies included the results related to natural conception rates and, interestingly, there is no data in the literature regarding the effects of HSG procedure performed with OSCM on ovarian stimulation cycles with gonadotropins as a frequently used method in infertility treatment.

As mentioned above, although improving fertility rates in the first four months after HSG have been indicated by previously published reports, the present authors hypothesized that the relationship was more important within the first month particularly for assisted reproductive technologies. In order to determine whether HSG procedures performed with OSCM have any impact on stimulated cycles with gonadotropins combined with IUI, the authors designed this retrospective study that stimulated IUI cycles performed in first six months after HSG procedure with lipiodol were compared according to HSG-IUI at one-month and longer than one-month intervals.

Materials and Methods

Study design and selected patients

The retrospective study was conducted in the Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology at Dokuz Eylul University School of Medicine, Izmir, Turkey. The study protocol was approved by the local Ethics committee, and informed consent was obtained from

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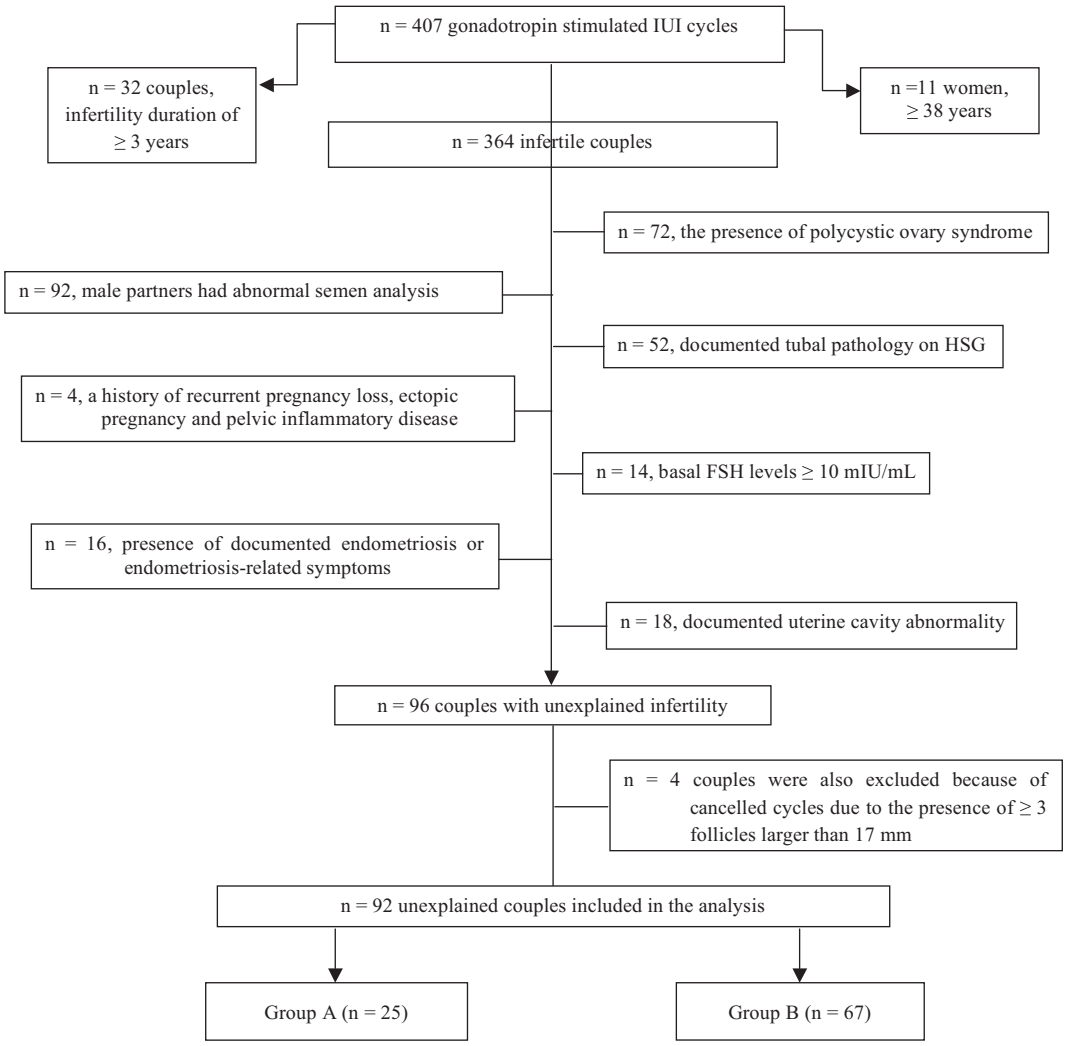

Figure 1. — Patients' flow charts.

all of the participants. Data were obtained between the periods January 2008 and May 2014 from a total of first IUI cycles of 407 infertile couples who had full medical records. All the IUI cycles were stimulated by gonadotropins after completing three clomiphene citrate cycles without pregnancy.

A standard infertility work-up consisting of detailed history, gynecologic examination, transvaginal sonography, and basal serum hormone profile evaluation including FSH, LH, TSH, and PRL on days between 2 and 5 of the cycle, HSG to determine tubal condition, serum P level measurement on the mid-luteal period, and semen analysis of their male partners were performed prior to the treatment of all participants. Semen analysis and its evaluation were done according to the World Health Organization (WHO) criteria and Kruger morphology [6]. To ensure homogeneity of the study group, studied group was limited only to women under age 38 with unexplained infertility, regular menstrual cycles, and duration of infertility ranging from one to three years. Exclusion criteria were as follows: 1) woman age ≥ 38 years (n = 11); 2) infertility duration period ≥ three years (n = 32); 3) presence of any documented tubal patency abnormalities on HSG (n = 52); 4) partners having abnormal semen analysis based on WHO criteria (WHO, 2010); and/or severe male factor infertility < 5 x 10^6 motile spermatozoa after sperm preparation as defined previously by a report of Saleh *et al.* [10] (n = 92 couples); 5) personal history of recurrent pregnancy loss, ectopic pregnancy, and pelvic inflammatory disease (n = 4); 6) presence of polycystic ovary syndrome which was diagnosed in accordance with criteria of Rotterdam Consensus [7] (n = 72); 7) previous ovarian surgery; determined or previously documented endometriosis or endometriosis-related symptoms including dysmenorrhea, dyspareunia and chronic pelvic pain (n = 16); 8) FSH ≥ ten mIU/ml on cycle day 2 to 5 based on a previous report [8] (n = 14); 9) presence of any documented uterine cavity abnormalities on HSG (n = 18); 10) HSG-IUI time interval longer than six months (Figure 1).

Finally, a total of 92 unexplained infertile couples undergoing their first cycle of IUI stimulated by gonadotropins were included in the analysis and they were classified into two groups according to IUI cycles performed one month (Group A, n = 25 cycles) or longer than one month (Group B, n = 67 cycles) after the HSG procedure (Figure 1).

HSG, ovarian stimulation, and IUI

HSG examinations were performed within the first five days after the end of menstruation, as described in detail by a previously published report [9]. Oil-based radio-opaque medium was

Table 1. — *Demographic and basal hormonal characteristics of the cases.*

Characteristics	Group A n = 25 (%)	Group B n = 67 (%)	*p* value
Women age (years)	29.1 ± 4.1	28.5 ± 3.7	NS
Male age (years)	31.6 ± 4.7	32.2 ± 4.7	NS
Body mass index (kg/m^2)	24.7 ± 4.7	24.9 ± 4.2	NS
Duration of infertility (years)	2.2 ± 0.5	2.4 ± 0.7	NS
Primary infertility (%)	20 (80)	61 (91)	NS
FSH (IU/L)	6.3 ± 1.2	6.1 ± 1.3	NS
LH (IU/L)	6.1 ± 1.6	6.5 ± 1.4	NS
Prolactin	20.2 ± 8.6	18.4 ± 7.8	NS
E2 (pg/ml)	34.7 ± 6.7	35.3 ± 7.2	NS
TSH (mIU/ml)	1.83 ± 0.44	1.72 ± 0.79	NS

Note: Values are means ± SD. NS = not significant.

slowly injected via a metal cannula under fluoroscopic control so as to show filling of the uterine cavity and bilaterally fallopian tubes and to obtain radiographic images.

All stimulations were done with exogenous gonadotropins including recombinant or highly purified human FSH and hMG. Gonadotropins were commenced at a dose between 50 to 150 IU/day on second or third day of the spontaneous menstruation and were given until the day of hCG administration. The gonadotropin starting dose was determined by a combination of factors including patient age and ovarian reserve testing including basal FSH measurements and antral follicle counts, and the doses were adjusted according to the patient's response which was monitored with serial transvaginal ultrasound assessment of the follicular development with endometrial thickness and pattern. When the mean diameter of at least one follicle had reached ≥ 18 mm, ovulation was triggered with 250 μg of recombinant hCG and IUI was planned approximately 36 ± 2 hours later.

The semen samples were collected into a sterile container at the laboratory after three to five days of sexual abstinence period. After 30 minutes of liquefaction at room temperature, the volume, concentration and motility were evaluated according to WHO criteria (WHO, 2010). The density gradient centrifugation and swim-up techniques were used to prepare the spermatozoa. The liquefied semen was centrifuged at 350 g for 15 minutes. Following discarding the supernatant, two milliliters of sperm washing medium was added and was re-centrifuged at 250 g for five minutes for two times. After removing the supernatant, 0.5 ml of sperm medium was covered over final pellet with caution and it was placed into the incubator for 30 to 45 minutes at 37°C with the angle of inclination kept constant. IUIs were done by using soft catheters with a one- or two-ml syringe and the patients remained in a supine position for 10–15 minutes after the procedure based on the results of a previous report [10]. Luteal supports were provided in all patients using vaginal progesterone gel once a day beginning two days after IUI until the 12th week of pregnancy if the patient conceived. Plasma β-HCG levels were measured two weeks after IUI and a serum β-HCG level > 50 mIU/ml was accepted as biochemical pregnancy. Clinical pregnancy was defined as transvaginal ultrasonographic visualization of an embryo with cardiac activity on sonography.

Statistical analysis

Data are presented as mean ± standard deviation. Statistical analysis was carried out using Chi-squared test or Fisher's exact test and the groups were compared by Student's *t*-test as appropriate. A $p < 0.05$ was considered statistically significant. Statistical analysis was performed with SPSS 15.0.

Table 2. — *Comparative evaluation of clinical parameters of stimulated IUI cycles which were performed one month or longer than one month after HSG in the unexplained infertile women.*

Characteristics	IUI cycles		*p* value
	Group A n = 25	Group B n = 67	
Duration of stimulation (days)	9.6 ± 2.6	10.1 ± 3.1	NS
Total gonadotropin dosage (IU)	795 ± 332.05	830.37 ± 346.23	NS
Number of follicles ≥ 18 mm	1.32 ± 0.47	1.35 ± 0.48	NS
Endometrial thickness on hCG day (mm)	9.7 ± 2.4	10.3 ± 1.7	NS
Sperm concentration (x10^6/ml)	57.16 ± 44.32	51.23 ± 35.97	NS
Total motile sperm count (TMSC) (x10^6)	80.90 ± 73.80	71.32 ± 57.62	NS
Progressive motility (%)	45.12 ± 17.47	44.34 ± 15.97	NS
TMSC after preparation (x10^6/ ml)	11.42 ± 7.48	10.32 ± 8.14	NS
Kruger morphology (%)	4.80 ± 1.46	4.19 ± 1.60	NS
Clinical pregnancy rate n (%)	11/25 (44)	12/67 (17.9)	0.012

Note: Values are means ± SD. NS = not significant.

Results

Ninety-two unexplained infertile couples (25.2 %) of the total 364 infertile couples who underwent their first gonadotropin stimulated IUI cycles during the study period were analyzed. Of these couples, 25 women (27.2 %) were in Group A, while remaining 67 women (72.8 %) were in Group B. As shown in Table 1, demographics and hormonal features were not different between the groups.

The cycle characteristics of 92 first IUI cycles combined with gonadotropin ovarian stimulation were presented in Table 2. After treatment, overall clinical pregnancy rate was found as 25.0% (23 clinical pregnancies / 92 cycles) for the entire cases. Also, in Group A, there were significantly higher clinical pregnancy rates compared to Group B (OR: 3.6; 95% CI, 1.3–9.8, $p = 0.012$); whereas comparable results were found for all clinical parameters of IUI cycles among the two groups.

Discussion

HSG is still commonly used as a diagnostic method in evaluation of uterine cavity and fallopian tubes as an important part of the work-up of female infertility. Also, there are some reports in the literature concerning predictive or therapeutic role of HSG in obtaining fertility. It has been reported that cumulative pregnancy rate was nearly 30% after HSG in natural cycles for one-year period and that

HSG was as valuable as laparoscopy in predicting fertility [11, 12]. These results are associated with treatment-free cycles and limited data was available about the prognostic and therapeutic capacity or effect of HSG for pregnancy on assisted reproductive cycles. One recent study has found that a normal HSG was sufficient as routinely performed laparoscopy on predicting the success of stimulated IUI cycles [13]. However, predictive factors for pregnancy in gonadotropin stimulated IUI cycles are well-described. In unexplained infertility, women's age < 38 years and duration of infertility < four years were considered as the most important prognostic factors for IUI cycles by large, well-designed reports [14-16]. In the present study, the participants were selected among unexplained couples who had good prognostic factors based on these criteria for homogenization of the study and this may also explain the relatively high pregnancy rates of presented study. As another important prognostic factor, the rates of women with secondary infertility were also comparable between the study groups.

HSG is performed with water soluble or oil soluble contrast agents and many studies have compared the effect of these substances on fertility outcomes. In a meta-analysis, it has been revealed that increased pregnancy rates after HSG were mainly associated with oil-soluble agents rather than water-soluble ones and the most pronounced increase was shown in unexplained couples [17]. Subsequently, tubal flushing as a treatment method for improving fertility has been widely investigated by numerous reports having conflicting results [3, 18]. In two randomized controlled studies dealing with this issue performed by the same workgroup, the authors have shown that tubal flushing with lipiodol, as an OSCM, had a positive impact on fertility rates in women with unexplained infertility and mild endometriosis [18, 19]. Also, they have demonstrated that this fertility improving effect of lipiodol was most prominent in the first six months after HSG [19]. Supporting this, a recent Cochrane analysis has confirmed that tubal flushing with OSCM had increased pregnancy rates in unexplained couples compared to those of both expectant management and one with water-soluble contrast media [3]. In another randomized controlled study, it has been determined that these increased pregnancy rates were particularly in the first six months after the procedure [4]. However, all of these results were on natural cycles and, interestingly, there is no data in the literature regarding the effects of oil-soluble contrast media on ovarian stimulation cycles with gonadotropins as a frequently used method in infertility treatment. To the present authors' knowledge, this is the first study in the literature investigating the effect of HSG with lipiodol on pregnancy rates after gonadotropin stimulated IUI cycles within the first six months following HSG in unexplained infertile couples. The present results also showed that the likelihood of achieving clinical pregnancy was 3.6 times (95% confidence interval 1.3–9.8, $p = 0.012$) higher for women who underwent IUI cycle within one month after HSG compared to the ones whose IUI cycles were performed later.

The effect of lipiodol on improving pregnancy rates of natural cycles has not been clearly understood yet, whereas several mechanisms have been proposed. The first proposed effect is mechanical and it might be associated with the discard of tubal debris and mucous plugs from tubal lumen or disruption of peritubular adhesions or lipiodol's positive impacts on tubal ciliary activity [1]. The second is immunologic that might be due to peritoneal macrophage modulation [3]. Finally, it has been suggested that there was an effect of increased endometrial receptivity following contrast media exposure [20]. The present results may also be associated with any of these mechanisms or a combination of all.

There are a number of limitations to this present study. The authors hypothesized that HSG with lipiodol, as in natural cycles, can have a positive effect on assisted reproductive techniques and it was intended to be a pilot study. The authors designed this retrospective study so the evidence requires confirmation with prospective studies. Some factors associated with IUI success were not analyzed such as sexual intercourse frequency, dietary or life-style features. Another main limitation is lack of laparoscopic assessment for all patients' evaluation. Although these limitations are often encountered in previous reports, the results of presented study should be confirmed by other studies.

In conclusion, in gonadotropin stimulated IUI cycles performed following one month after HSG with lipiodol procedure, there seems to be an improvement in pregnancy rates in unexplained couples. In unexplained cases, it may be a reasonable approach to plan IUI cycles in the first month after HSG in clinical practice.

References

[1] Dun E.C., Nezhat C.H.: "Tubal factor infertility: diagnosis and management in the era of assisted reproductive technology". *Obstet. Gynecol. Clin. North Am.,* 2012, *39*, 551.

[2] Schankath A.C., Fasching N., Urech-Ruh C., Hohl M.K., Kubik-Huch R.A.: "Hysterosalpingography in the workup of female infertility: indications, technique and diagnostic findings". *Insights Imaging*, 2012, *3*, 475.

[3] Luttjeboer F., Harada T., Hughes E., Johnson N., Lilford R., Mol B.W.: "Tubal flushing for subfertility". *Cochrane Database Syst. Rev.,* 2007, *3*, CD003718.

[4] Nugent D., Watson A.J., Killick S.R., Balen A.H., Rutherford A.J.: "A randomized controlled trial of tubal flushing with lipiodol for unexplained infertility". *Fertil. Steril.,* 2002, *77*, 173.

[5] Saunders R.D., Shwayder J.M., Nakajima S.T.: "Current methods of tubal patency assessment". *Fertil. Steril.,* 2011, *95*, 2171.

[6] Kruger T.F., Acosta A.A., Simmons K.F., Swanson R.J., Matta J.F., Oehninger S.: "Predictive value of abnormal sperm morphology in in vitro fertilization". *Fertil. Steril.,* 1988, *49*, 112.

[7] Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group: "Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome". *Fertil.*

Steril., 2004, *81*, 19.
[8] Miller P.B., Acres M.L., Proctor J.G., Higdon H.L. 3rd., Boone W.R.: "Flexible versus rigid intrauterine insemination catheters: a prospective, randomized, controlled study". *Fertil. Steril.,* 2005, *83*, 1544.
[9] Baramki T.A.: "Hysterosalpingography". *Fertil. Steril.,* 2005, *83*, 1595.
[10] Saleh A., Tan S.L., Biljan M.M., Tulandi T.: "A randomized study of the effect of 10 minutes of bed rest after intrauterine insemination". *Fertil. Steril.,* 2000, *74*, 509.
[11] Verhoeve H.R., Coppus S.F., van der Steeg J.W., Steures P., Hompes P.G., Bourdrez P., *et al.*: "The capacity of hysterosalpingography and laparoscopy to predict natural conception". *Hum. Reprod.,* 2011, *26*, 134.
[12] den Hartog J.E., Lardenoije C.M., Severens J.L., Land J.A., Evers J.L., Kessels A.G.: "Screening strategies for tubal factor subfertility". *Hum. Reprod.,* 2008, *23*, 1840.
[13] Tanahatoe S.J., Lambalk C.B., Hompes P.G.: "The role of laparoscopy in intrauterine insemination: a prospective randomized reallocation study". *Hum. Reprod.,* 2005, *20*, 3225.
[14] Merviel P., Heraud M.H., Grenier N., Lourdel E., Sanguinet P., Copin H.: "Predictive factors for pregnancy after intrauterine insemination (IUI): an analysis of 1038 cycles and a review of the literature". *Fertil. Steril.,* 2010, *93*, 79.
[15] Jeon Y.E., Jung J.A., Kim H.Y., Seo S.K., Cho S., Choi Y.S., Lee B.S.: "Predictive factors for pregnancy during the first four intrauterine insemination cycles using gonadotropin". *Gynecol. Endocrinol.,* 2013, *29*, 834.
[16] Hill M.J., Whitcomb B.W., Lewis T.D., Wu M., Terry N., DeCherney A.H., Levens E.D., Propst A.M.: "Progesterone luteal support after ovulation induction and intrauterine insemination: a systematic review and meta-analysis". *Fertil. Steril.,* 2013, *100*, 1373.
[17] Watson A., Vandekerckhove P., Lilford R., Vail A., Brosens I., Hughes E.: "A meta-analysis of the therapeutic role of oil soluble contrast media at hysterosalpingography: a surprising result?" *Fertil. Steril*., 1994, *61*, 470.
[18] Johnson N.P., Farquhar C.M., Hadden W.E., Suckling J., Yu Y., Sadler L.: "The FLUSH trial--flushing with lipiodol for unexplained (and endometriosis-related) subfertility by hysterosalpingography: a randomized trial". *Hum. Reprod.,* 2004, *19*, 2043.
[19] Johnson N.P., Kwok R., Stewart A.W., Saththianathan M., Hadden W.E., Chamley L.W.: "Lipiodol fertility enhancement: two-year follow-up of a randomized trial suggests a transient benefit in endometriosis, but a sustained benefit in unexplained infertility". *Hum. Reprod.,* 2007, *22*, 2857.
[20] Johnson N.P.: "Review of lipiodol treatment for infertility - an innovative treatment for endometriosis-related infertility?" *Aust. N. Z. J. Obstet. Gynaecol.,* 2014, *54*, 9.

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