

Effect of Sonohysterography on Assisted Reproductive Technology (ART) Success Rates in Infertile Women :A Randomized Clinical Trial

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Received: 5th Jan, 2026; Revised: 25th Jan 2026; Accepted: 20th Feb, 2026; Available Online: 10th Mar, 2026

ABSTRACT

Since implantation failure is one of the reasons for ART failure, various strategies have been proposed to improve pregnancy rates. One recommended method is inducing endometrial injury. This study aimed to evaluate the effect of sonohysterography on ART success rates in infertile women. A total of 300 infertile women who had indications for ART were randomly assigned to either the sonohysterography group or the control group. The first group, sonohysterography was performed on days 19-21 of the cycle. Both groups then underwent ART using a standard long GnRH agonist protocol. 12-14 days after embryo transfer, BHCG was checked in both groups; if positive, sonography was performed 3 weeks after embryo transfer to evaluate the presence of a gestational sac. Finally, patients were followed to determine and compare ART success rates between the two groups. Among the 300 infertile women who met the inclusion criteria, the mean age in the sonohysterography and control groups was 31.3 ± 3.9 and 32.6 ± 3.5 , respectively ($P=0.14$). There was no statistical difference between the two groups in terms of the number of oocytes, the number of embryos, or the grade of embryos. The mean endometrial thickness in the sonohysterography group was higher than in the control group (8.08 ± 1.04 vs. 7.27 ± 1.13 ; $P=0.0001$). The BHCG positivity rate was 43.3% in the sonohysterography group and 24.7% in the control group ($P=0.001$). ART success, (observation of a gestational sac), was 42% in the sonohysterography group and 20% in the control group. Sonohysterography prior to ART was associated with improved pregnancy rates. As a minimally invasive and cost-effective procedure, it may be considered as supportive approach to enhance endometrial receptivity.

Keywords: Infertility, Sonohysterography, ART.

How to cite this article: Heidar Z, Daraei M, Hazari V, Saadat F, Sadraei SA. Effect of sonohysterography on assisted reproductive technology (ART) success rates in infertile women: a randomized clinical trial. *Int J Drug Deliv Technol.* 2026;16(72s): 119-124. DOI: 10.25258/ijddt.16.72s.16

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Assisted Reproductive Technology (ART) includes all methods that directly manipulate oocytes outside the body [1]. In modern assisted reproductive techniques, the technology related to embryo culture and transfer is developing and advancing. At the same time, during this time, the pregnancy rate has not changed significantly, and many patients suffer from embryo implantation failure [2]. For the doctor and his patient, the most important factor of frustration in IVF treatment is repeated implantation failure following the transfer of a good-quality embryo [3]. The success rate of IVF depends on the investigation of the infertility factor and the type of infertility treatment. Failure in IVF can be associated with various reasons, some of which are due to an unexplained underlying factor. Repeated failure in IVF is a very stressful situation for the doctor and the patient. The causes of these recurrent failures can be grouped into the following: factors related to

endometrial non-receptivity (uterine environment disorders, inappropriate endometrial thickness, lack of expression of adhesion molecules, immunological diseases and thrombophilia); factors related to incomplete fetal development (genetic disorders of the couple, gamete or fetus; increased thickness of the zona pellucida); multifactorial causes (endometriosis, presence of hydrosalpinx). Among these disorders, uterine factors can be diagnosed and treated through hysteroscopic methods [4].

Implantation, a necessary part of any successful pregnancy, can occur in a prepared uterus. In humans, the uterus is receptive to the embryo during the middle phase of the menstrual cycle (days 19-23). It is estimated that about two-thirds of implantation failures are due to inadequate uterine receptivity. Although many causes of infertility are reversible with IVF, implantation remains a critical stage [5]. Implantation is defined as the attachment of the embryo to the endometrium and subsequent invasion of the uterine

wall stroma. This process is complex, involving several steps in cytokine expression and close interaction between the embryo and endometrial tissue [6]. Since implantation failure is a high risk factor, various methods have been proposed to improve pregnancy rates. One of the most recommended methods is to induce mucosal injury in the endometrium. This relationship was first investigated in laboratory animals. Decidual formation and uterine receptivity for implantation were higher in animals with damaged endometrial mucosa. A similar response was reported following oil injection into the uterus of mice [7, 8].

In 1971, Karow and colleagues found that two of 28 women treated with luteal phase biopsy had a miscarriage, while the rest achieved clinical pregnancy. They hypothesized that endometrial injury enhanced the decidual response [9]. Two decades later, Friedler and colleagues treated 14 women with recurrent failure to conceive with a protocol of hysteroscopy, curettage, and antibiotics and estrogen, of whom 6 became pregnant. The authors suggested that recurrent failure was due to endometritis, which resolved with antibiotics. Estrogen administration also improves endometrial blood flow. Since it was part of the curettage protocol, it has been suggested that mucosal injury may also play a role in implantation [10]. The possible role of endometrial injury in improving implantation was first emphasized by Barash in 2003. They treated 45 women with more than one implantation failure with endometrial injury in the cycle before IVF stimulation and found that pregnancy rates improved. They stated that by inducing endometrial injury, the decidual response increased, thereby improving implantation. In this study, mucosal injury was induced using endometrial biopsy tubes on days 8, 12, 21, and 26 before the IVF cycle. Finally, the implantation, clinical pregnancy, and live birth rates in the control group were 28%, 67%, and 49%, respectively, and in the control group were 14%, 30%, and 23%, respectively [11].

Gnainsky and colleagues have shown in their previous research that performing an endometrial biopsy in a cycle more than IVF can double the implantation, clinical fertility, and live birth rates. This favorable effect has also been shown in other studies following endometrial injury. The authors hypothesized that the improvement in pregnancy rates following endometrial mucosal injury is due to the induction of an inflammatory response. In fact, high levels of TNF α , LIF, and IL6 occur in the early stages of implantation. Conversely, the absence of tissue response and dendritic cells in the decidua reduces implantation rates [12]. In a 2010 study by Ms. Gnainsky, which included 64 women candidates for IVF, the patients were divided into two groups. In the control group, a mucosal injury was performed on days 8-9 of the cycle. Then, both groups underwent a biopsy of the endometrial mucosa on days 21-23 of the cycle, and then IVF was performed. In this study, higher levels of inflammatory cytokines and a greater number of dendritic cells were observed in the mucosa of the control group (previous biopsy). Also, the rate of pregnancy outcomes was directly and positively correlated with the level of these cytokines and the expression of dendritic cells [5].

In a study conducted by Raziel and colleagues, endometrial injury in the luteal phase on days 21 and 26 was performed on 60 women with prior IVF treatment failure, and the

treatment outcomes were compared with those of 57 patients who did not undergo endometrial injury. In the injured group, implantation and pregnancy rates were reported as 30% and 11%, respectively, which were significantly higher than those in the control group (8% and 4%) [13]. Zhou and colleagues, by performing ultrasound-guided endometrial injury on days 22–25 of the ovarian stimulation cycle, concluded that inducing such injury is associated with increased pregnancy rates [14]. In Demirel's study of 421 women with more than two previous IVF failures, participants were divided into Group I (211 women), who did not undergo hysteroscopy, and Group II (210 women), who did undergo hysteroscopy. Group II was further divided into subgroups IIa (normal hysteroscopic findings) and IIb (abnormal hysteroscopic findings). Pregnancy success rates in groups IIb, IIa, and I were 21.6%, 32.5%, and 30.4%, respectively, showing a statistical difference between Groups II and I. However, no significant difference was observed between subgroups IIa and IIb. The difference between Groups I and II appeared to be related to the hysteroscopy procedure itself [15]. In 2011, Huang and colleagues investigated the effect of localized endometrial injury on pregnancy rates. They created a 10–15 mm injury at the posterior endometrium on days 4–7 of the ovarian stimulation cycle in six patients and observed that all patients achieved a positive pregnancy result. The posterior uterine site was chosen because previous studies indicated that embryo implantation within 10–20 mm of the uterine fundus offers the highest chance of pregnancy in IVF patients [2].

In a 2007 study by Nastri and colleagues in Brazil, 591 patients across five studies were evaluated for the effect of endometrial injury on pregnancy rates. In four studies, injury was performed 1 month before the treatment cycle, and in one study, injury was performed on the day of oocyte retrieval. Results showed that endometrial injury before the cycle increases pregnancy and live birth rates, whereas injury on the oocyte retrieval day is not recommended, as it may reduce pregnancy rates [16]. In a 2009 study by Karimzadeh and colleagues in Yazd, the effect of endometrial injury induced by a sampling catheter on improving implantation and pregnancy rates in patients with recurrent implantation failure undergoing ART was examined. Among 115 patients with at least 2 prior failures, participants were randomly assigned to 2 groups. In the case group, endometrial biopsy was performed during the luteal phase of the preceding cycle, and implantation and clinical pregnancy rates were assessed. The results indicated implantation rates of 10.9% in the case group versus 3.38% in the control group, and clinical pregnancy rates were significantly higher in the case group (27.1% vs. 8.9%) [17]. Rana Karayalcin and colleagues in Turkey (2012) investigated the effect of diagnostic hysteroscopy on pregnancy rates in IVF patients. A total of 1,258 patients were divided into three groups: Group 1 (407 patients) underwent hysteroscopy 50 days before embryo transfer; Group 2 (280 patients) underwent hysteroscopy 50 days to 6 months before transfer; and Group 3 (571 patients) underwent hysteroscopy more than 6 months before transfer. Implantation rates were 22.1%, 16.1%, and 11.1%, respectively; overall pregnancy rates were 48.2%, 38.9%, and 29.9%; and clinical pregnancy rates were 45.2%, 34.3%, and 27.1%, respectively. They concluded that performing

hysteroscopy 50 days or less before embryo transfer maximizes its effect on the endometrium and significantly improves successful pregnancy rates [18]. In a 2008 London review, Tarek and colleagues examined the effect of outpatient hysteroscopy on IVF outcomes in 1,691 IVF candidates. Patients were divided into two groups: those who underwent hysteroscopy and those who did not. Pregnancy rates were 42.6% in the hysteroscopy group versus 22.1% in the non-hysteroscopy group. They concluded that outpatient hysteroscopy before the treatment cycle can effectively increase pregnancy rates [19].

In 2012, Potdar conducted a systematic review and meta-analysis to assess the effect of endometrial injury before embryo implantation. The study indicated that mechanical injury of the endometrium via biopsy, curettage, or hysteroscopy in the cycle preceding ovarian stimulation for IVF improves implantation in patients with recurrent miscarriage. Potdar and colleagues reviewed all studies where patients underwent biopsy, curettage, or hysteroscopy before ovarian stimulation and considered the clinical pregnancy rate as treatment success. They analyzed seven studies (four randomized, three non-randomized) involving 2,062 patients and found that localized endometrial injury increased the likelihood of clinical pregnancy by 70% compared to no injury. Clinical pregnancy rates following biopsy/curettage were approximately twice as high as those following hysteroscopy (RR=2.32 vs. RR=1.51). This review provides strong evidence that localized endometrial injury can improve pregnancy outcomes in women with recurrent implantation failure. Such injury can be performed through superficial curettage via biopsy catheter or endoscopic uterine evaluation [20].

In patients requiring ICSI due to male infertility, the female partner is often not fully evaluated. Modern and advanced transvaginal ultrasound techniques provide an inexpensive, simple, and non-invasive evaluation of the uterine cavity and pelvic organs. The uterine cavity is a potential space that can be assessed by filling it with a sonographically visible fluid. Using this simple concept, the uterus can be distended with normal saline and evaluated with ultrasound with high precision. Sonohysterography is a simple, clinic-performable technique that does not require advanced radiologic equipment [21].

Various studies have shown that avoiding blood, mucus, bacterial contamination in the cervical canal, excessive uterine contractions, and endometrial trauma during embryo transfer are associated with increased implantation and pregnancy rates. One of the most recommended methods to improve pregnancy rates is inducing endometrial injury. Previous research has concluded that performing diagnostic hysteroscopy at the beginning of the treatment cycle changes the endometrial environment through trauma-induced inflammation, improves endometrial blood flow, and ultimately enhances implantation success [18]. However, hysteroscopy is costly and invasive. If sonohysterography can similarly impact the uterine cavity environment, enhance endometrial function and receptivity, or induce mild endometrial trauma to improve implantation and pregnancy rates, it can be used as a low-cost, accessible, complication-free outpatient procedure [22]. Additionally, this method allows verification of uterine cavity health. Based on this, the

present study aimed to investigate the effect of sonohysterography on ART success at the beginning of the treatment cycle in infertile women referring to Mahdiah Hospital, Tehran, during 2012–2013.

MATERIALS AND METHODS

This clinical trial enrolled patients referred to the Mahdiah Hospital Infertility Center in Tehran. In this interventional study, which was conducted as a single-blind randomized clinical trial, patients who referred to the Mahdiah Hospital Infertility Center during 2012 and 2013, had an indication for an ART cycle, were willing to participate in the study, and completed a written consent form were included. The study population included infertile women who were candidates for ART and were referred to the Mahdiah Hospital Infertility Center in Tehran during 2012 and 2013. 300 infertile women who were candidates for ART (based on a pilot study and a review of articles and literature) were selected as a sample, with a sample size of 150 for each group. Patients who were referred to the Mahdiah Hospital Infertility Center, had an indication for an ART cycle, were willing to participate in the study, and completed a written consent form were included.

The inclusion criteria included all patients with indications for ART (including age factor, tubal factor, male factor, ovarian reserve disorder, and unexplained). Exclusion criteria included underlying systemic diseases, infectious diseases, patients who develop ovarian hyperstimulation syndrome during the cycle, and patients who have undergone hysteroscopy in the last three months. A total of 300 infertile women were randomly assigned to one of two groups (150 in each group) based on a random number table. Patients in the case group underwent sonohysterography on days 19-21 of the cycle. To perform sonohysterography, the patients' vaginas were first disinfected with 10% betadine solution. In the next step, a pediatric Foley catheter was passed through the cervical canal and placed into the uterine cavity. Then, CC 20 (Sodium chloride 0.9%, Darupaksh) was slowly injected into the uterus, and, simultaneously with the injection, the internal cavity of the uterus, the adnexa around the ovary, and the cul-de-sac were examined with a vaginal probe placed in the posterior fornix of the vagina.

Then, treatment was started in both groups using the Long Protocol method with GnRH agonist (superfact). The HMG dose used (merional, IPSA, 75 UI) was determined based on the patient's age and the protocol approved by the infertility department. 5 days later, a control ultrasound was performed. If at least 3 follicles measuring 17-18mm were seen, 2 units of HCG 5000 UI (choriomon) were injected, and the endometrial thickness was also measured at this time. 36 hours later, a puncture was performed, and 2 to 3 days later, embryo transfer was performed. 12-14 days after embryo transfer, BHCG was checked in both groups; if positive, ultrasound was performed 3 weeks after embryo transfer to evaluate the presence of a gestational sac.

Finally, patients were followed up to obtain ART results, and the ART success rate in the two groups was determined and compared. Success was defined as a positive pregnancy test and observation of a gestational sac on transvaginal sonography. The treating physician was unaware of the patient's group during the gonadotropin stimulation cycle and puncture.

Data analysis was performed using SPSS version 18.

RESULTS

A total of 300 infertile women who met the inclusion criteria remained in the study through 2012 and 2013, and their data were included in the final assessment. The mean ($\pm$ SD) age of the patients was 31.8 ± 5.8 years (range 18 to 45 years). Table 1 summarizes the baseline characteristics of the study participants. The mean age (years) in the sonohysterography and control groups was 31.3 ± 5.9 and 32.3 ± 5.6 years, respectively ($P=0.14$). There was no significant difference in the number of oocytes between the two groups ($P=0.3$). The mean number of oocytes in the sonohysterography group was 10.2 ± 5.3 (1-25), and in the control group was 9.9 ± 4.8 (1-25). The number of embryos transferred to the mothers' uterus in both groups was statistically similar ($P=0.07$). In the sonohysterography group, the average number of embryos was 3.1 ± 1.1 , and in the control group, 2.8 ± 0.8 .

The grade of embryos transferred to the mothers' uterus was divided into two groups: favorable (A and B) and unfavorable (C and D). In the sonohysterography group, a favorable embryo grade was transferred in 139 cases (92.7%) and an unfavorable grade in 11 cases (7.3%). In the control group, a favorable embryo grade was transferred in 147 cases (98%) and an unfavorable grade in 3 women (2%). These rates were not statistically different in both groups ($P=0.8$).

The average endometrial thickness (mm) in the sonohysterography and control groups was 8.08 ± 1.04 mm and 7.27 ± 1.13 mm, respectively. Based on this, it can be said that the endometrial thickness in the sonohysterography group is statistically greater than that in the control group ($P=0.0001$). The BHCG test was reported positive in 65 patients (43.3%) in the sonohysterography group and negative in 85 patients (56.7%). The BHCG test was reported positive in 37 patients (24.7%) in the control group and negative in 113 patients (75.3%). The rate of positive BHCG test results in the sonohysterography group was higher than in the control group, and the difference was statistically significant ($P=0.001$). In those with a positive BHCG test, transvaginal ultrasound was performed, and in the sonohysterography group, a gestational sac was observed in 63 women (42%), and in the control group, in 31 patients (20.8%). The rate of gestational sac observation in the sonohysterography group was statistically higher ($P=0.0001$).

Given that the success rate of ART is the same as the visualization of the gestational sac by transvaginal ultrasound, it can be concluded that the success rate of ART following sonography in women is twice as high as in the group in which this procedure was not performed (42% vs. 20%).

Table 1. Comparison of demographic characteristics and treatment outcomes between the two groups.

Variable	Sonohysterogra phy Group	Contr ol Group	P- value
Age (years)	31.3 ± 5.9	32.6 ± 5.6	0.14
Number of oocytes	10.2 ± 5.3 (8) 12	9.9 ± 4.8	0.3

<4 4-12 >12	(62.6) 94 (29.4) 44	(11.3) 17 (69.4) 104 (19.3) 29	
Number of embryos	3.1 ± 1.1	2.8 ± 0.8	0.07
Embryo Grade (%) Favorable (A & B) Unfavorab le (C & D)	139 (92.7%) 11 (7.3%)	143 (95.3 %) 7 (4.7%)	0.8
Endometri al Thickness (mm) <7 ≥ 7	8.08 ± 1.04 (8) 12 (92) 138	7.27 ± 1.13 (38.7) 58 (61.3) 92	0.000 1
BHCG Status (%) Positive Negative	65 (43.3%) 85 (56.7%)	37 (24.7 %) 113 (75.3 %)	0.001
Gestationa l Sac (%) Positive Negative	63 (42%) 87 (58%)	31 (20.8 %) 118 (79.2 %)	0.000 1
ART Success (%)	42	20.8	0.000 1

DISCUSSION AND CONCLUSIONS

Embryo implantation, which is essential for any successful pregnancy, can only occur in a prepared uterus. This process is highly complex and involves multiple stages, including cytokine expression and close interactions between embryonic and endometrial tissues. Due to the high likelihood of implantation failure, various strategies have been proposed to improve pregnancy rates. One of the most recommended methods is inducing endometrial injury. The relationship between endometrial injury and implantation was first investigated in laboratory animals. It was observed that the formation of decidua and uterine receptivity for implantation were higher in animals that had endometrial injury. A similar reaction was reported following intrauterine injection of oil in mice. The potential role of endometrial injury in improving implantation was first highlighted by Barash in 2003. In their study, 45 women with more than one previous implantation failure underwent endometrial injury in the cycle before IVF stimulation, and an improvement in pregnancy rates was observed. They suggested that endometrial injury increases the decidual reaction, which in turn enhances implantation [11]. In Gnainsky's study, patients who underwent endometrial

biopsy showed higher levels of inflammatory cytokines and a greater number of dendritic cells in the endometrium. Moreover, pregnancy outcomes were directly and positively correlated with the levels of these cytokines and dendritic cell expression [5]. In 2012, Potdar, in a systematic review and meta-analysis, reported that mechanical injury to the endometrium via biopsy, curettage, or hysteroscopy in the cycle before ovarian stimulation for IVF improves embryo implantation in patients with recurrent miscarriage [20]. Numerous studies investigating the effect of endometrial biopsy and hysteroscopy on ART success have generally concluded that inducing endometrial lesions increases the chance of uterine receptivity for implantation.

However, limitations such as the need for surgery, anesthesia risks, and high costs hinder the universal application of hysteroscopy. Sonohysterography, in contrast, is a simple, outpatient-performable technique that does not require advanced radiologic equipment or anesthesia. It can be performed with high accuracy, is cost-effective, and helps evaluate uterine abnormalities.

Based on these observations, we conducted the present study to investigate the efficacy of sonohysterography in improving fertility outcomes in women undergoing ART. In our study, endometrial thickness in the sonohysterography group was statistically higher than in the control group. This suggests that the improvement in pregnancy rates following endometrial injury may be due to the induction of an inflammatory reaction, which increases endometrial thickness in patients. In the present study, the gestational sac was observed in 42% of women in the sonohysterography group compared to 20% in the control group. The success rate of this method is comparable to other studies that have used endometrial biopsy or hysteroscopy to improve fertility outcomes.

It is recommended that the results of the present study be further evaluated with larger sample sizes and by examining the physiopathological effects of the procedure.

Author contributions:

Zahra Heidar: Conceptualization ,Methodology and Supervision

Maedeh Daraei: data collection, Data analysis, Investigation and Writining-original draft

Vajiheh Hazari: Writining-review and editing

Fargol Saadat: Data collection, procedure performance

Azadeh sadraei: Data collection, procedure performance

Funding: This research was funded by the Infertility Research Center at Shahid Beheshti University

Conflict of interest: The authors declare that they have no conflict of interest.

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Figure 2. Embryo grade distribution in the sonohysterography and control groups.

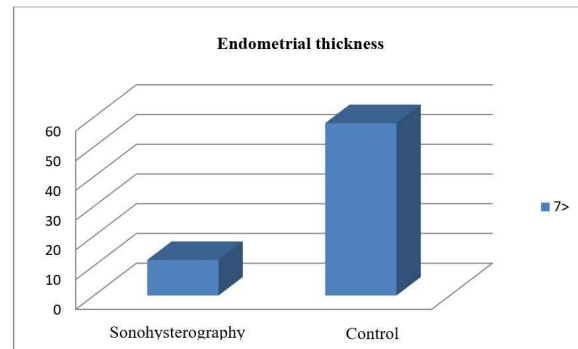


Figure 3. Endometrial thickness in the sonohysterography and control groups.

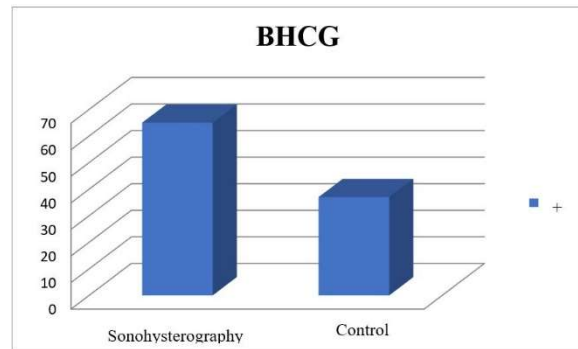


Figure 4. Rate of positive BHCg test in the sonohysterography and control groups.

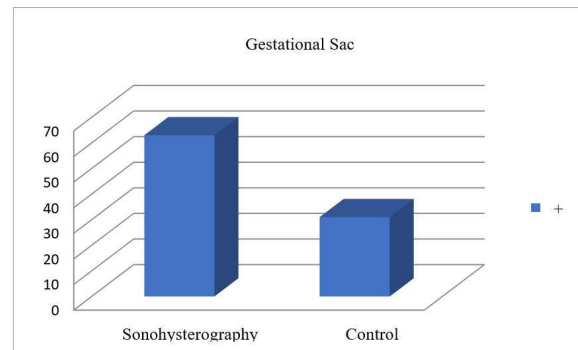


Figure 5. Rate of gestational sac observation in the sonohysterography and control groups.

Figure captions

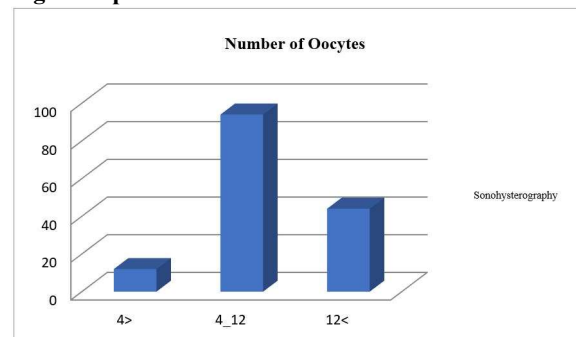


Figure 1. Number of oocytes formed in the sonohysterography and control groups.

