



Vaginal Three-Dimensional Ultrasound and Chromohysteroscopy for Endometrial Evaluation in Cases of Failed Intracytoplasmic Sperm Injection

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Implantation failure could be due to a variety of reasons, including embryo quality and uterine receptivity, but remains unexplained in many cases. The aim of the work was to evaluate vaginal three-dimensional ultrasound, chromohysteroscopy for endometrial evaluation in cases of previous failed intracytoplasmic sperm injection and histopathological examination of the suspected areas.

Methods: This study was carried on 50 patients who attended to the department of Gynecology in Tanta University Hospital and another private hospital. All patients was allocated in one group and examined between 30 and 120 days after the failed ICSI cycle. 3D vaginal ultrasound was done for all cases at the early luteal phase period then all the hysteroscopic operations was performed in the early follicular phase (3-4 days postmenstrual) as conventional hysteroscopy.

Results: Most common hysteroscopic finding was Polypoidal endometrium in 12% of cases, Arcuate uterus in 10% of cases, Arcuate uterus + T shaped cavity in 8% of cases and Niche, Polypectomy in 4% of cases and Biopsy of dark stained area was taken from all cases. Histopathology finding was normal Proliferative endometrium in 72% of cases and Early secretory endometrium in 4% of cases. Focal chronic nonspecific endometritis in 20% of cases and Polypoid endometrial hyperplasia in 4%.

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Conclusions: Chromohysteroscopy is a safe and easy procedure which can help to improve the efficacy of hysteroscopy in diagnosing cases of endometritis and to increase the success rate of IVF and ICSI. It is highly recommended to be applied to cases of repeated ICSI or IVF failure.

Keywords: *Three-dimensional ultrasound; vaginal; chromohysteroscopy; endometrial; intracytoplasmic sperm injection.*

1. INTRODUCTION

In-vitro fertilization (IVF) is an expensive treatment but results in a successful outcome in only a third of treatment cycles [1].

Implantation failure could be due to a variety of reasons, including embryo quality and uterine receptivity, but remains unexplained in many cases. It represents a major cause of stress to both the clinician and the patient undergoing intracytoplasmic sperm injection (ICSI) cycle. Even minor uterine cavity abnormalities, such as endometrial polyps, small submucous myomas, adhesions, and septa are considered to have a negative impact on the chance to conceive [2].

The presence of uterine pathology may negatively affect the chance of implantation. The prevalence of unsuspected uterine pathology in asymptomatic women with implantation failure has been reported to be as high as 50% [3].

The high prevalence of intrauterine pathologies in infertile women makes evaluation of the uterine cavity for fibroids, polyps, adhesions, and Mullerian abnormalities a reasonable decision. Therefore, one of the common investigations proposed for women undergoing IVF treatment is to evaluate the uterine cavity via hysteroscopy. Hysteroscopy is the gold standard test for assessing the uterine cavity [4].

It is generally performed as a definitive diagnostic tool to evaluate abnormal findings on hysterosalpingogram or saline hysterosonography performed during the course of investigation of subfertile women [5].

Hysteroscopy not only provides accurate visual assessment of the uterine cavity, but also provides a chance to treat any pathology detected during the examination. The availability of hysteroscopes with a smaller diameter has made the use of outpatient or office hysteroscopy feasible as a routine examination [6].

Currently, there is evidence that performing hysteroscopy before starting IVF treatment could

increase the chance of pregnancy in the subsequent IVF cycle in women who had one or more failed IVF cycles [7].

The aim of the work was to evaluate Vaginal three-dimensional ultrasound, hromohysteroscopy for endometrial evaluation in cases of previous failed intracytoplasmic sperm injection and histopathological examination of the suspected areas.

2. PATIENTS AND METHODS

This study was carried on 50 patients who attended to the department of Gynecology in Tanta University Hospital and another private hospital. Patients aged between 20 and 35 years with primary infertility, body mass index 18-30 kg/m², tubal factor of infertility except hydrosalpinx, male factor of infertility, unexplained infertility, failed ICSI cycle in spite of good quality embryos, normal function of the uterus according to hysterosalpingography and hysteroscopy or transvaginal ultrasonography were included in the study.

While patients aged > 35 years with body mass index < 30 kg/m², detected uterine anatomical abnormality, cancelled cycles for not having any embryos to transfer, evidence of Aschermann's syndrome, hyperprolactinemia and medical disease e.g., Thyroid, Renal, liver disease, hypertension were excluded from the study.

All patients was allocated in one group and examined between 30 and 120 days after the failed ICSI cycle. 3D vaginal ultrasound was done for all cases at the early luteal phase period then all the hysteroscopic operations was performed in the early follicular phase (3-4 days postmenstrual) as conventional hysteroscopy.

All patients were subjected to the following: verbal and written consent, detailed clinical history, general and local clinical examination, laboratory investigations: FSH,LH, Anti Mullerian Hormone (AMH), Prolactin and thyroid stimulating hormone were measured for all patients in their previous cycles, vaginal

ultrasound and history of previous ICSI cycles, number of embryos transferred, quality of embryos, and presence of any associated pelvic pathology that was present during the ICSI cycles (hydrosalpinx, endometriosis).

3D vaginal ultrasound was done for all cases at the early luteal phase period then all the hysteroscopic operations was performed in the early follicular phase (3-4 days postmenstrual) as conventional hysteroscopy and documentation of the findings was done. The 2.9 mm, 30° rigid telescope with an operative sheath of 5.3 mm was used during examination.

All cases was given 1 gm ceftriaxone IV immediately before hysteroscopy. All cases was given the hysteroscopy as an outpatient procedure in infertility clinic in Tanta University Hospital. Chromohysteroscopy: in this procedure, five millimeters of 1% sterile methylene blue dye was introduced through embryo transfer catheter (Labotect). Immediately after dye injection, the catheter was removed and introduction of the hysteroscope was done. The distension media flow was start again (normal saline) and let wash of the endometrium from the dye. The uterine cavity was examined for the staining pattern. Diffuse light blue staining was considered normal. Focal, dark blue staining above the internal cervical ostium regardless of the size and number of the stained areas, was considered positive finding. Hysteroscopic guided biopsy was done by hysteroscopic scissors, fixed in 10% neutral buffered formalin and was sent to a pathology center in for histopathological examination. Paraffin embedded blocks was prepared and sections 3-5 µm in thickness was obtained and subjected to H&E staining. The corner stone in our study was assessing the endometrial cavity condition by 3D ultrasound & Chromohysteroscopy, presence of endometritis and other occult pathologies as a suspected cause for ICSI failure.

3. STATISTICAL ANALYSIS

Statistical analysis was done by SPSS v26 (IBM Inc., Chicago, IL, USA). Quantitative variables were presented as mean and standard deviation (SD). Qualitative variables were presented as frequency and percentage (%). Quantitative non-parametric data were presented as median and interquartile range (IQR). A receiver operating characteristic curve, or ROC curve, is a graphical plot that illustrates the diagnostic ability of a binary classifier system as its discrimination

threshold is varied. For abnormally distributed data, Sensitivity, specificity, positive and negative predictive values and diagnostic accuracy were estimated.

4. RESULTS

The age and BMI of studied patients are shown in Table 1.

The mean duration of infertility was 3.53 years and common cause was Ovulatory problem in 36% of cases, Male factor in 30% of cases, Tubal factor in 24% of cases, Endometriosis in 36% of cases and Unexplained infertility in 4%. [Table 2].

The mean days of menstrual cycle were 6.38, mean number of failed cycles were 1.52 and mean number of embryos transferred were 2.02. The mean AMH level was 2.89, mean FSH was 7.5, mean LH level was 5.8, mean TSH was 3.73 and mean PRL was 15.12. [Table 3].

Ultrasound had normal findings in 48% cases, and most common abnormality founded was Cs scar niche in 18% of cases followed by, Arcuate uterus in 10% of cases, Arcuate uterus + T shaped cavity in 8 %, Cervical echogenic mass q polyp, Endometrial polyp, Polypoidal endometrium in 4% of cases, Cervical polyp, False Endometrial polyp in 2%. Most common hysteroscopic finding was Polypoidal endometrium in 12% of cases, Arcuate uterus in 10% of cases, Arcuate uterus + T shaped cavity in 8% of cases and Niche, Polypectomy in 4% of cases and Biopsy of dark stained area was taken from all cases. Histopathology finding was normal Proliferative endometrium in 72% of cases and Early secretory endometrium in 4% of cases, Focal chronic nonspecific endometritis in 20% of cases and Polypoid endometrial hyperplasia in 4%. [Table 4].

Sensitivity, specificity, PPV, NPV and accuracy of Ultrasound finding in diagnosing intra uterine pathology in comparison to histopathology was 50%, 47.22%, 26.92%, 70.83%, 48% respectively and there were significant differences between ultrasound findings in comparison to Histopathology. Sensitivity, specificity, PPV, NPV and accuracy of Hysteroscopic finding in diagnosing intra uterine pathology in comparison to histopathology was 14.29%, 77.78%, 20%, 70%, 60% respectively. [Table 5].

Table 1. Distribution of the studied cases according to age and BMI (n = 50)

	No.	%
Age (years)		
≤30	35	70.0
>30	15	30.0
Min. – Max.	25.0 – 36.0	
Mean ± SD.	29.10 ± 3.45	
Median (IQR)	28.0 (26.0 – 32.0)	
BMI (kg/m²)		
Min – Max	21.0 – 30.0	
Mean ± SD	24.38 ± 2.83	
Median (IQR)	24.0 (23.0 – 24.0)	

Table 2. Distribution of the studied cases according to causes and duration of infertility(n = 50)

	No.	%
Causes of infertility		
Male factor	15	30.0
Ovulatory problem	18	36.0
Endometriosis	3	6.0
Tubal factor	12	24.0
Unexplained infertility	2	4.0
Duration of infertility		
Min. – Max.	2.0 – 5.0	
Mean ± SD.	3.53 ± 0.83	
Median (IQR)	3.40 (3.0 – 4.0)	

Table 3. Descriptive analysis of the studied cases according to different parameters (n = 50)

	Min. – Max.	Mean ± SD.	Median (IQR)
Days of menstrual cycle	4.0 – 9.0	6.38 ± 1.26	6.0(5.0 – 7.0)
Number of failed cycles	1.0 – 3.0	1.52 ± 0.68	1.0(1.0 – 2.0)
Number of embryos transferred	1.0 – 3.0	2.02 ± 0.55	2.0(2.0 – 2.0)
	Min. – Max.	Mean ± SD.	Median (IQR)
AMH (ng/mL)	1.0 – 3.60	2.89 ± 0.44	3.0(2.80 – 3.10)
FSH (IU/L)	6.0 – 9.80	7.50 ± 1.10	7.40(6.7 – 7.8)
LH level (IU/ml)	3.50 – 10.0	5.80 ± 1.27	5.80(4.70 – 6.70)
TSH	2.90 – 4.80	3.73 ± 0.66	3.55(3.3 – 4.3)
PRL	10.0 – 17.0	15.12 ± 1.22	15.0(14.0 – 16.0)

SD: Standard deviation, IQR: Inter quartile range

Table 4. Distribution of the studied cases according to ultrasound finding and Hysteroscopic finding (n = 50)

Ultrasound finding	No.	%
Normal	24	48.0
Cs scar niche	9	18.0
Cervical echogenic mass q polyp	2	4.0
Arcuate uterus	5	10.0
Arcuate uterus + T shaped cavity	4	8.0
Endometrial polyp	2	4.0
False Endometrial polyp	1	2.0
Polypoidal endometrium	2	4.0
Cervical polyp	1	2.0

Hysteroscopic finding	No.	%
Biopsy of dark stained area	50	100.0
Polypoidal endometrium	6	12.0
Niche	2	4.0
Polypectomy	2	4.0
Arcuate uterus	5	10.0
Arcuate uterus + T shaped cavity	4	8.0
Histopathology	No.	%
Proliferative endometrium	36	72.0
Focal chronic nonspecific endometritis	10	20.0
Early secretory endometrium	2	4.0
Polypoid endometrial hyperplasia	2	4.0

Table 5. Agreement (sensitivity, specificity and accuracy) Ultrasound finding in diagnosing intra uterine pathology in comparison to histopathology (n = 50) and Hysteroscopic finding in diagnosing intra uterine pathology in comparison to histopathology (n = 50)

	Histopathology		Sensitivity	Specificity	PPV	NPV	Accuracy
	Normal (n = 36)	Abnormal (n = 14)					
	No.	%	No.	%			
Ultrasound finding							
Normal	17	47.2	7	50.0	50.0	47.22	26.92 70.83 48.0
Abnormal	19	52.8	7	50.0			
χ² (p)	0.031 (0.860)						
	Histopathology		Sensitivity	Specificity	PPV	NPV	Accuracy
	Normal (n = 36)	Abnormal (n = 14)					
	No	%	No	%			
Hysteroscopic finding							
Normal	28	77.8	12	85.7	14.29	77.78	20.0 70.0 60.0
Abnormal	8	22.2	2	14.3			
χ² (FE p)	0.397 (0.704)						

χ²: Chi square test, FE: Fisher Exact p: p value for association between different categories, PPV: Positive predictive value, NPV: Negative predictive value

5. DISCUSSION

In-vitro fertilization (IVF) is an expensive treatment but results in a successful outcome in only a third of treatment cycles [8]. Implantation failure could be due to a variety of reasons, including embryo quality and uterine receptivity, but remains unexplained in many cases [9].

In the current study we found that ultrasound had normal findings in 48% cases, and most common abnormality founded was Cs scar niche in 18% of cases followed by, Arcuate uterus in 10% of cases, Arcuate uterus + T shaped cavity in 8 %, Cervical echogenic mass q polyp, Endometrial polyp, Polypoidal endometrium in 4% of cases, Cervical polyp,

False Endometrial polyp in 2%. Kandeel MA et al [2] showed that the abnormal uterine cavity is detected in about 25% of cases evaluated by TVS. In Khalifa EA et al [10] Ultrasound findings 21 (84%) had normal ultrasound findings, 1 (4%) had acute AVF and 3 (12%) had RVF. In the control group, 22 (88%) had normal ultrasound findings and 3 (12%) had RVF.

In the current study we found that most common hysteroscopic finding was Polypoidal endometrium in 12% of cases, Arcuate uterus in 10% of cases, Arcuate uterus + T shaped cavity in 8% of cases and Niche, Polypectomy in 4% of cases and Biopsy of dark stained area was taken from all cases. Gupta T et al. [11] showed that Diagnostic hysteroscopy revealed endometrial polyps in 10, submucous fibroids in 8 and ulcerative lesion in 1 out of 50 cases. Marconi et al, Kucuk and Safali

[12,13] reported that the endometrium is not an absorptive epithelium in normal circumstances and that structural damage of the cells allows passage of methylene blue dye into the cells.

Another study by Küçük T and Safali M [13] showed that conventional hysteroscopy and chromohysteroscopy procedure was successful in all 63 patients. Group II included 41 patients in whom diffuse light blue staining was observed. Histopathologic examination revealed nine cases of endometritis in group I (40.9%). On the other hand, four cases of endometritis was diagnosed histopathologically with random biopsy in group II (9.7%) [14].

In the current study we found that histopathology finding was normal Proliferative endometrium in 72% of cases and Early secretory endometrium in 4% of cases, Focal chronic nonspecific endometritis in 20% of cases and Polypoid endometrial hyperplasia in 4%.

Vijay A et al [15] showed that the histopathological examination showed normal finding in 40% and hormonal imbalance in 34% of cases. It is important to note that, all cases of complex endometrial hyperplasia with atypia and endometrial carcinoma showed focal staining. La Sala et al [16] reported an incidence of 2% endometritis among 100 women with two consecutive IVF failures. Incidence of endometritis in our series was higher. That must be related to better targeting of biopsy by endometrial dying. Another study by Khalifa EA et al [10] showed that presence of endometritis in 8 (32%) out of the patients had no presence of endometritis (diffuse light staining) and 17 (68%) had presence of endometritis (focal dark staining).

In the current study we found that sensitivity, specificity, PPV, NPV and accuracy of Ultrasound finding in diagnosing intra uterine pathology in comparison to histopathology was 50%, 47.22%, 26.92%, 70.83%, 48% respectively and there were significant differences between ultrasound findings in comparison to Histopathology p-value 0.031.

As regard efficacy of TVUS in diagnosing endometrial pathology Maiti G et al found that Sensitivity and specificity of TVUS in diagnosing endometrial polyp was 57.5%, 100% respectively, submucous fibroid Sensitivity and specificity was 65.38%, 100% respectively, EHP in postmenopausal bleeding

Sensitivity and specificity was 75%, 100 % respectively, endometrium carcinoma sensitivity and specificity was 50%, 100% respectively. Hysteroscopy showed sensitivity and specificity of Hysteroscopy in diagnosing endometrial polyp was 93.3%, 100% respectively, submucous fibroid sensitivity and specificity was 100%, 100% respectively, EHP in postmenopausal bleeding sensitivity and specificity was 75%, 100 % respectively, endometrium carcinoma sensitivity and specificity was 50%, 100% respectively [17].

Compared with hysteroscopically guided biopsy, TVUS has a sensitivity of 19 to 96%, a specificity of 53 to 100%, a positive predictive value of 75 to 100% and a negative predictive value of 87 to 97% for diagnosing endometrial polyps.

According to guidelines by the AAGL, SHG has a sensitivity ranging from 58 to 100%, a specificity ranging from 35 to 100%, a PPV range of 70 to 100% and a NPV range of 83 to 100%, compared with hysteroscopically guided biopsy. Adding saline contrast solution to 3D sonography increased the specificity to 88 to 99% and the PPV to 97 to 100% for endometrial polyps, compared with 3D ultrasonography (sensitivity of 92% to 95% and NPV of 97%) [18].

In the current study we found that sensitivity, specificity, PPV, NPV and accuracy of Hysteroscopic finding in diagnosing intra uterine pathology in comparison to histopathology was 14.29%, 77.78%, 20%, 70%, 60% respectively. Another study by Küçük T and Safali M [14] showed that hysteroscopy was a significant predictor of miscarriage after IVF-ET with the sensitivity, specificity, positive and negative predictive values were 54%, 99%, 94% and 89% respectively. Paschopoulos et al [19] reported sensitivity and specificity of 92% and 95% for hysteroscopy in diagnosing intra-cavitary pathology like submucosal myoma and endometrial polyp, in women with abnormal uterine bleeding.

Allameh et al [20] reported very high diagnostic accuracy of hysteroscopy. He found 93% sensitivity and 100% specificity for detection of endometrial polyps and 100% sensitivity and 96.4% specificity for submucosal fibroid. Another study by Gupta T et al [11] showed that the diagnostic accuracy of chromohysteroscopy in evaluation of endometrial pathology was 86.67% with sensitivity of 11/12 = 91.67%, specificity of

41/48 = 85.41%, PPV of 11/18 = 61.11% and NPV of 41/42 = 97.61%.

Hysteroscopy showed sensitivity, specificity, NPV, and PPV of 94.2%, 88.8%, 96.3%, and 83.1%, respectively, in predicting normal or abnormal histopathology of endometrium. Highest accuracy was in diagnosing endometrial polyps, with sensitivity, specificity, NPV, and PPV of 95.3%, 95.4%, 98.9%, and 81.7%, respectively; the worst result was in estimating hyperplasia, with respective figures of 70%, 91.6%, 94.3%, and 60.6%.

All failures of hysteroscopic assessment resulted from poor visualization of the uterine cavity or from underestimation or overestimation of irregularly shaped endometrium [21].

In Towbin et al [22] in their study of efficacy of TVS versus hysteroscopy in evaluation of uterine cavity in patients with excessive uterine bleeding found hysteroscopy to be significantly more sensitive as compared to TVS. They studied 149 cases of menorrhagia, metrorrhagia and postmenopausal bleeding and found sensitivity of 54% with TVS and 79% with hysteroscopy. Specificity of the two investigative modalities, however, was comparable in their study. Moreover, on comparing TVS results with hysteroscopy-guided biopsy, Nadin A [23] found that TVS in the diagnosis of polyp has a sensitivity, specificity, and accuracy of 20, 100, and 80%, respectively. TVS in the diagnosis of myoma has a sensitivity, specificity, and accuracy of 100, 80, and 85%, respectively. Both TVS and hysteroscopy have a sensitivity of 100%, a specificity of 100%, and an accuracy of 100%. A second point of valuable importance was the agreement achieved by both hysteroscopy-guided biopsy and ultrasound in detecting endometrial lesions and the nonagreement in detecting myometrial lesions. Other investigators, while dealing with similar parameters to detect the value of sonography in relation to hysteroscopy in diagnosing intracavitary uterine pathology, found that sensitivity, specificity, positive, and negative likelihood ratios were 67%, 87%, 5.15, and 0.38 for TVS and 92%, 95%, 18.4, and 0.08 for hysteroscopy [24]. In another study, 50 patients with abnormal uterine bleeding were studied by Dijkhuizen et al [25] using TVS and saline infusion sonography (SIS). Histological examination revealed normal endometrial histology in 27 patients, submucous myomas in 13 patients, and intracavitary polyps in 10

patients. The sensitivity of TVS in directly visualizing intracavitary abnormalities was 61%, with a specificity of 96%. The sensitivity and specificity of SIS was 100 and 85%. No intracavitary abnormality was missed by SIS. In a similar study by De Vries et al [26] who studied 62 patients, TVS demonstrated 60% sensitivity. The likelihood ratio of the presence of an intracavitary abnormality was 8 and the likelihood ratio of the absence of an intracavitary abnormality was 0.43. In routine practice, TVS and HSG are the main tools to document the uterine texture prior to ART; however, the diagnostic accuracies of these are quite low with limited sensitivities and specificities [27]. Diagnostic limitation is likely to be more prominent in those with a history of RIF, as the frequency of unrecognized pathologies may be up to 50% [28, 29]. In the study by Gao et al. [30] nearly 80% of intrauterine abnormalities were found to be undiagnosed with HSG or TVS in those with RIF. This rate is approximately 50% in our data. As all the women in the office hysteroscopy (oHS) arm underwent their first oHS procedure in our study, the prevalence of abnormalities was considered to be relatively high.

Endometrial polyps are quite common and have been shown to compromise pregnancies, depending on the size, by interfering with embryo implantation [31]. It has been shown that polypectomy prior to IVF, even for small polyps (<2 cm), might improve the take-home baby rate in patients undergoing IVF [32]. Thus, the routine removal of polyps prior to a new ART attempt is also suggested [31]. Despite previous data underlining the beneficial impact of correcting unsuspected uterine cavity abnormalities, a very recent TROPY trial failed to demonstrate such an impact [33]. This might be explained with the fact that they identified cervical or uterine cavity abnormalities only in 26% of women and two-thirds of those were not treated. In our data, apparent polyps and adhesions were the most common findings, and 40% of the women in the oHS group were treated. It has been shown that the fertility-enhancing effect of oHS could also be independent from the correction of intrauterine abnormalities. Hysteroscopy has been proposed to improve ART outcomes through an endometrial injury process leading to embryo implantation [34].

In a recent meta-analysis, endometrial scratching (four studies) or oHS (three studies) was shown to increase the CPRs of women with a history of

RIF when induced in the preceding cycle of OS [35]. The same favorable results have been reported when oHS was performed within 50 days [27] or even within 6 months prior to a new ART [30]. On the other hand, this impact is somehow questionable in light of the recent conflicting evidence [36]. Further research is still needed to optimize instrumentation and timing.

6. CONCLUSION

Chromohysteroscopy is a safe and easy procedure which can help to improve the efficacy of hysteroscopy in diagnosing cases of endometritis and to increase the success rate of IVF and ICSI. It is highly recommended to be applied to cases of repeated ICSI or IVF failure.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

It was approved by the ethical committee of Tanta University hospitals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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