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Endometrial Evaluation in Failed Intracytoplasmic Sperm Injection by Vaginal Three-Dimensional Ultrasound and Chromo-Hysteroscopy

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Abstract:

Objectives: The research aimed to assess the effectiveness of intracytoplasmic sperm injection in the past, chromo-hysteroscopy for endometrial assessment, and histological investigation of suspicious locations.

Method: This Observational study ,Fifty patients. After the unsuccessful intracytoplasmic sperm injection (ICSI) cycle, all patients were placed in a single cohort and evaluated between 30 and 120 days later. All patients underwent 3D vaginal ultrasonography during the early luteal phase, and all hysteroscopic procedures were carried out as conventional hysteroscopy during the early follicular phase (three to four days postmenstrual).

Results: 72% of patients had normal proliferative endometrium, 4% had early secretory endometrium, 20% had localized chronic nonspecific endometritis, and 4% had polypoidal endometrial hyperplasia, according to histopathology. Polypoidal endometrium was seen in 12% of cases, followed by arcuate uterus in 10% of cases, arcuate uterus + T-shaped cavity in 8% of instances, Niche, polypectomy in 4% of cases, and biopectomy of a darkly stained region in all cases.

Conclusion: Chromo-hysteroscopy is a straightforward and risk-free method that can improve the accuracy of hysteroscopy in identifying endometritis and increasing the success rate of vitro fertilization (IVF) and ICSI. It is strongly advised to be administered in situations of recurrent ICSI or IVF failure.

Keywords: Three-Dimensional Ultrasound, Vagina, Chromo Hysteroscopy, Endometrial, Intracytoplasmic Sperm Injection.

Introduction

In vitro fertilization (IVF) is a costly procedure that results in a successful outcome in only one-third of treatment cycles, despite the fact that it is an expensive treatment **Chiware et al.,(2020)**.

Implantation failure can be caused by a variety of reasons, the exact nature of which is sometimes uncertain. These factors include embryo quality and uterine receptivity. It is a significant source of anxiety for the patient enduring an intracytoplasmic sperm injection (ICSI) cycle as well as the treating physician. It is believed that certain abnormalities in the uterine cavity, including as small submucous myomas, adhesions, septa, and endometrial polyps, can reduce the chances of conception **Kandeel et al.,(2020)**.

The likelihood of implantation may be adversely affected by uterine disease. It has been observed that up to 50% of asymptomatic women experiencing implantation failure have undetected uterine disease **Cenksoy et al.,(2013)**.

One of the common tests recommended for women undergoing in vitro fertilization is a hysteroscopy examination of the uterine cavity due to the high prevalence of intrauterine illnesses in infertile women. This is because hysteroscopy is the gold standard examination for evaluating the uterus. **Pundir and El Toukhy,(2010)**, and it is usually carried out as the final diagnostic method to assess anomalous results on a saline or hysterosalpingogram. Hysterosonography is carried out when researching infertile ladies. **Anand et al.,(2020)**. In addition to providing a precise visual assessment of the uterine cavity, hysteroscopy offers the chance to treat any disease discovered during the process. **Campo et al.,(2018)**. There is currently evidence that, for women who have had one or more unsuccessful IVF cycles, undergoing a hysteroscopy before initiating IVF therapy may enhance the likelihood of becoming pregnant in the subsequent IVF cycle **Mao et al.,(2019)**.

Evaluation of vaginal three-dimensional ultrasonography, chrome-hysteroscopy for endometrial evaluation in situations of prior intracytoplasmic sperm injection failure, and histological investigation of the suspicious regions were the objectives of the work.

Materials and Methods

This Observational study involved 50 patients who visited the gynecology department at Tanta University Hospital and another private hospital. Patients with primary infertility, body mass index between 18 and 30 kg/m², tubal factor of infertility (except hydrosalpinx), male factor of infertility, unexplained infertility, failed ICSI cycle despite high-quality embryos, and normal uterine function as determined by hysterosalpingography, hysteroscopy, or transvaginal ultrasonography were included in the study. Patients older than 35 years old who had detected uterine anatomical abnormality, canceled cycles because they had no embryos to transfer, evidence of Aschermann's syndrome, hyperprolactinemia, and medical conditions such as thyroid, renal, and liver disease were not included in the study. After the unsuccessful ICSI cycle, all patients were placed in a single cohort and evaluated between 30 and 120 days later. The study was done after approval from the Ethical Committee faculty of medicine of Tanta University (approval code: 32434/07/18).

All patients underwent 3D vaginal ultrasonography during the early luteal phase, and all hysteroscopic procedures were performed as conventional hysteroscopy during the early follicular phase (three to four days postmenstrual). Verbal and written consent, a thorough clinical history, a general and local clinical examination, laboratory investigations (including measuring FSH, LH, anti-Mullerian hormone (AMH), prolactin, and thyroid stimulating hormone for each patient in their prior cycles), vaginal ultrasound and the history of prior ICSI cycles, the quantity and quality of embryos transferred, and the presence of any associated pelvic pathology that was present during the ICSI cycles (hydrosalpinx, endometriosis) were all administered to all patients. All patients had 3D vaginal ultrasonography during the early

luteal phase, and all hysteroscopic procedures, including conventional hysteroscopy, were carried out during the early follicular phase (three to four days postmenstrual). Documentation of the results was also completed. Examinees employed a stiff telescope measuring 2.9 mm by 30° with an operating sheath of 5.3 mm. Before hysteroscopy, 1 gm of ceftriaxone IV was administered to each patient. The hysteroscopy was administered to each patient as an outpatient treatment at Tanta University Hospital's infertility center. Chromato hysteroscopy: Using an embryo transfer catheter (Labotect), five millimeters of 1% sterile methylene blue dye were inserted during this technique. The hysteroscope was introduced and the catheter was withdrawn right after following the dye injection. Restarting the distension media flow with regular saline allowed the dye to wash out of the endometrium. The staining pattern inside the uterus was investigated. It was accepted that diffuse light blue staining was typical. A positive outcome was defined as focused, dark blue staining over the interior cervical ostium, regardless of the size or number of stained patches. Using hysteroscopic scissors, a hysteroscopic guided biopsy was performed. The sample was then preserved in 10% neutral buffered formalin and forwarded to a pathology center for histological analysis. Blocks fixed in paraffin were made, and slices ranging in thickness from 3 to 5 µm were obtained and stained with H&E.

The fundamental component of our research was evaluating the state of the endometrial cavity using 3D ultrasound and chromo hysteroscopy, as well as looking for endometritis and other occult diseases as potential reasons for ICSI failure.

Statistical analysis:

IBM Inc., Chicago, IL, USA used SPSS v26 for statistical analysis. The standard deviation (SD) and mean were used to represent quantitative variables. Qualitative factors were shown as percentages (%) and frequencies. The interquartile range (IQR) and median were employed to display quantitative non-parametric data. An ROC curve is a graphical representation of the relationship between a binary classifier system's discriminating threshold and its diagnostic

accuracy. Data with an irregular distribution was tested for diagnostic accuracy, positive and negative predictive values, specificity, sensitivity, and specificity.

Results

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

The mean duration of infertility was 3.53 years, and the 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 the menstrual cycle were 6.38, the mean number of failed cycles was 1.52 and a mean number of embryos transferred was 2.02. The mean AMH level was 2.89, the mean FSH was 7.5, the mean LH level was 5.8, the mean TSH was 3.73 and the mean PRL was 15.12. **(Table 3)**

The distribution of the studied cases according to ultrasound findings and Hysteroscopic findings are shown in **Table 4**.

Sensitivity, specificity, PPV, NPV, and accuracy of Ultrasound findings in diagnosing intrauterine pathology in comparison to histopathology were 50%, 47.22%, 26.92%, 70.83%, and 48% respectively and there were significant differences between ultrasound findings in comparison to Histopathology. Sensitivity, specificity, PPV, NPV, and accuracy of Hysteroscopic findings in diagnosing intrauterine pathology in comparison to histopathology were 14.29%, 77.78%, 20%, 70%, and 60% respectively. **(Table 5)**

Discussion

IVF is only successful in around one-third of treatment cycles **Margalioth et al.,(2006)**. One of the common tests recommended for women undergoing in vitro fertilization is a hysteroscopy examination of the uterine cavity due to the high prevalence of intrauterine illnesses in infertile women. This is because hysteroscopy is the gold standard examination for

evaluating the uterus. **Pundir and El Toukhy,(2010)**, and it is usually carried out as the final diagnostic method to assess anomalous results on a saline or hysterosalpingogram.

The current study showed that there were significant differences between ultrasound findings and histopathology, with a p-value of 0.031. The study also revealed that the sensitivity, specificity, PPV, NPV, and accuracy of ultrasound findings in diagnosing intrauterine pathology were, respectively, 50%, 47.22%, 26.92%, 70.83%, and 48%.

Regarding TVUS's effectiveness in identifying endometrial disease, **Maiti et al.,(2018)** Discovered that its sensitivity and specificity for identifying endometrial polyps were, respectively, 57.5% and 100%, submucous fibroid Sensitivity and specificity in postmenopausal bleeding were 65.38% and 100%, respectively, according to EHP. Endometrial cancer has a sensitivity and specificity of 75% and 100%, respectively. Specificity and sensitivity were 100% and 50%, respectively. Endometrial polyps and submucous fibroids were diagnosed with a sensitivity of 93.3% and a specificity of 100% using hysteroscopy. When it came to postmenopausal hemorrhage, the sensitivity and specificity were both 100%, whereas when it came to endometrial cancer, they were 75% and 100%, respectively. 50 percent for sensitivity and 100 percent for specificity.

When accessible, the AAGL recommended that TVUS be the investigation of choice for identifying endometrial polyps since it offers trustworthy information in this regard. TVUS produces the most precise results during the menstrual cycle's proliferative phase. Comparing TVUS to hysteroscopically guided biopsy, the former has a sensitivity of 19 to 96%, specificity of 53 to 100%, positive predictive value of 75 to 100%, and negative predictive value of 87 to 97% for endometrial polyp detection. TVUS's sensitivity, specificity, PPV, and NPV are 86%, 94%, 91%, and 90%, respectively. In symptomatic patients, Power Doppler raises TVUS sensitivity to 95%. Greyscale TVUS with color-flow Doppler may have higher specificity and NPV—up to 95% and 94%, respectively. According to the AAGL guidelines,

as compared to hysteroscopically guided biopsy, SHG has a sensitivity range of 58% to 100%, a specificity range of 35% to 100%, a PPV range of 70% to 100%, and an NPV range of 83% to 100%.

Sonography, with or without 3D imaging, is more effective in identifying endometrial polyps when intrauterine contrast is added. Multiple studies found no statistically significant difference between hysteroscopy and SHG for the detection of endometrial polyps. SHG has the benefit of being able to evaluate tubal patency in addition to the uterine cavity and other pelvic structures.

The drawbacks include a lengthier learning curve compared with TVUS, an inability to diagnose endometrial disease, and increased patient pain. When compared to two-dimensional TVUS, studies using non-contrast 3D TVUS demonstrated little improvement in diagnostic capacity (sensitivity of 100%, specificity of 71% to 99%, PPV of 89% to 99%, and NPV of 100%).

The sensitivity of 3D ultrasonography was 92% to 95% and the NPV was 97% when contrast fluid was injected instead of saline. In the case of endometrial polyps, this raised the PPV to 97-90% and the specificity of the imaging to 87-90% ,(2012). In the current investigation, we discovered that the hysteroscopic finding's sensitivity, specificity, PPV, NPV, and accuracy in identifying intrauterine disease was 14.29%, 77.78%, 20%, 70%, and 60%, respectively, when compared to histology.

It has a resemblance to the research conducted by Küçük T and Safali M. **Küçük and Safali,(2008)**, which demonstrated that chromohysteroscopy was more accurate in identifying endometrial diseases.

According to separate studies by Küçük T and Safali M **Küçük and Safali,(2008)**, hysteroscopy significantly increased the risk of miscarriage during IVF-ET. The study's positive, negative, and sensitivity predictive values were, in order, 54%, 99%, 94%, and 89%.

When women have irregular menstrual flow, **Pasqualotto et al.,(2000)** Observed that hysteroscopy had a 92% and 95% sensitivity and specificity in identifying intra-cavitary diseases such as submucosal myoma and endometrial polyp.

Hysteroscopy's extremely high diagnostic accuracy was reported by **Allameh and Mohammadizadeh,(2007)** They discovered that there was 100% sensitivity and 96.4% specificity for submucosal fibroid and 93% sensitivity and 100% specificity for endometrial polyps.

Gupta et al.,(2019) found that chromo hysteroscopy had a diagnostic accuracy of 86.67% when evaluating endometrial illness. The specificity was 85.41%, PPV was 61.11%, and NPV was 97.61%. The sensitivity was 91.67%, the specificity was 41/48, and the PPV was 11/18. When it came to determining whether endometrial histology was normal or abnormal, hysteroscopy had a sensitivity of 94.2%, specificity of 88.8%, NPV of 96.3%, and PPV of 83.1%. The diagnosis of endometrial polyps had the highest accuracy, with sensitivity, specificity, NPV, and PPV of 95.3%, 95.4%, 98.9%, and 81.7%, respectively; the estimation of hyperplasia had the lowest accuracy, with corresponding values of 70%, 91.6%, 94.3%, and 60.6%.

All hysteroscopic evaluation failures were caused by inadequate uterine cavity visualization or by overestimating or underestimating the unevenly shaped endometrium. **Garuti et al.,(2001)**. Hysteroscopy was shown to be much more sensitive than TVS in Towbin et al.'s research. **Towbin et al.,(1996)** Evaluate the uterine cavity in patients with severe uterine hemorrhage using TVS vs hysteroscopy.

After analyzing 149 cases of menorrhagia, metrorrhagia, and postmenopausal bleeding, they discovered that hysteroscopy had a 79% sensitivity while TVS had a 54% sensitivity. However, in their investigation, the two investigative techniques' specificity was equivalent. Furthermore, Nadin A ^{Alaa},(2012) discovered that TVS had a sensitivity, specificity, and accuracy of 20, 100,

and 80%, respectively, in the identification of polyps when comparing its results with those from hysteroscopy-guided biopsy.

When it comes to diagnosing myomas, TVS provides sensitivity of 100, specificity of 80, and accuracy of 85%. The sensitivity, specificity, and accuracy of both TVS and hysteroscopy are 100%. A second important discovery was that ultrasonography and hysteroscopy-guided biopsy agreed to detect endometrial lesions but disagreed to identify myometrial lesions. Similar criteria were employed by other researchers to assess the relative merits of sonography and hysteroscopy in the identification of intracavitary uterine disease. They found that the values for hysteroscopy were 92%, 95%, 18.4, and 0.08, while for TVS, the values were 67%, 87%, 5.15, and 0.38 in terms of sensitivity, specificity, positive, and negative probability ratios.

Paschopoulos et al.,(2001). TVS and saline infusion sonography (SIS) were used by **Dijkhuizen et al.,(2000)** In another investigation to examine 50 individuals with unexplained uterine hemorrhage.

Following a histological investigation, 27 individuals had normal endometrial histology, 13 had submucous myomas, and 10 had intracavitary polyps.

With a specificity of 96%, TVS has a sensitivity of 61% for directly visualizing intracavitary anomalies. With SIS, the sensitivity was 100% and the specificity was 85%. SIS failed to detect any intracavitary abnormalities. TVS showed 60% sensitivity in comparable research by De Vries et al. **de Vries et al.,(2008)** Which examined 62 patients. The probability ratio indicating the existence of an intracavitary abnormality was 8, whereas the probability indicating its absence was 0.43. The specificity of TVS was 21% and sensitivity was 85% when the endometrial thickness was more than 5 mm or when an intracavitary abnormality was directly visible; the likelihood ratios for these two criteria were 1.1 and 0.71, respectively.

The probability ratios for the presence and absence of intracavitary anomalies for SIS were 0.13, 10; 95%; 88%; and specificity; respectively. The primary methods used in ordinary

practice to record the uterine texture before ART are TVS and HSG; nevertheless, their diagnostic accuracy is rather low, with limited sensitivity and specificity **Karayalçin et al.,(2012)**. Given that up to 50% of undiagnosed diseases may occur in people with a history of RIF, diagnostic restriction is probably more common in these individuals. **Bozdag et al.,(2008and Makrakis et al.,(2009)**.

About 80% of intrauterine anomalies in patients with RIF were not detected using HSG or TVS, according to research by Gao et al. **Gao et al.,(2015)**. Our analysis indicates that this rate is around 50%. Given that every woman in the office hysteroscopy (OHS) arm of our research had undergone an OHS procedure for the first time, the prevalence of abnormalities was deemed to be rather elevated. Unless OHS is done before a new cycle, it appears that a sizable portion of RIF patients are mistakenly classified as having "normal" uterine texture for a while. Despite typical findings on TVS or HSG in RIF instances, all the provided data support the necessity for OHS.

Due to their tendency to obstruct embryo implantation, endometrial polyps are quite prevalent and have been demonstrated to jeopardize fertility, depending on their size. **Coughlan et al.,(2014)**. Research has shown that removing polyps (small growths less than 2 cm) prior to in vitro fertilization (IVF) will improve the success rate of taking a baby home with IVF patients **Lass et al.,(1999)**. Therefore, it is also advised too routinely Remove Polyps Before Starting a New ART effort **Coughlan et al.,(2014)**.

A recent trial, TROPHY, failed to show any benefit from treating unanticipated uterine cavity defects, despite prior results supporting this. **El-Toukhy et al.,(2016)**. This may be explained by the fact that only 26% of the women had abnormalities in the uterus or cervical cavity detected and that two-thirds of those women did not get treatment.

Forty percent of the women in the OHS group got therapy, and the most common outcomes were visible adhesions and polyps. Research indicates that the impact of OHS on fertility may

potentially exist independently of the treatment of intrauterine anomalies. It has been suggested that hysteroscopy, which causes endometrial damage before implantation, might enhance the results of ART. **Kilic et al.,(2013)**.

This influence is hypothesized by the fact that, in our data, the outcomes of the women in the OHS arm who had no pathologic findings were typically better than those of the women who did not have OHS. Abdel-Qader et al. **Abdel-Qader et al.,(2024)** Found that the normal hysteroscopy subgroup's results were significantly better than those of the controls in their meta-analysis (RR=1.63, 95% CI 1.35–1.98, $p<0.001$). There is also a chance that the amount of time between mechanical damage and ET will affect the course of a pregnancy. A recent meta-analysis found that women with a history of RIF experienced higher CPRs when endometrial itching or OHS were triggered in the prior cycle of OS, according to four studies and three studies, respectively.

Potdar et al.,(2012). The same favorable results have been demonstrated when OHS was performed within 50 days **Karayalçin et al.,(2012)** or even within 6 months prior to beginning a new antiviral treatment **Gao et al.,(2015)**. This impact, however, is somewhat questionable in light of the current contradictory results **Panagiotopoulou et al.,(2015)**. Timing and instrumentation optimization still require more study.

However, the study had some limitations as the small sample size, its single-center design, and lack of a control group.

Conclusion

A simple and safe technique called chromohysteroscopy can enable hysteroscopy to diagnose endometritis more accurately and boost the success rate of IVF and ICSI. It is strongly advised to be used in situations when ICSI or IVF treatments repeatedly fail.

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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)

SD: standard deviation, IQR: interquartile range. BMI: body mass index.

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 $\pm$ SD.	3.53 $\pm$ 0.83
Median (IQR)	3.40 (3.0 – 4.0)

SD: standard deviation, IQR: interquartile range.

Table 3: Descriptive analysis of the studied cases according to different parameters

(n = 50)	Min. – Max.	Mean ± SD.	Median (IQR)
Days of the 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. AMH: anti-Mullerian hormone.

Table 4: Distribution of the studied cases according to ultrasound findings and hysteroscopic findings

Ultrasound finding (n = 50)	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 findings in diagnosing intrauterine pathology in comparison to histopathology (n = 50) and Hysteroscopic findings in diagnosing intrauterine 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%)					
χ^2 (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%)					
χ^2 (^{FE} p)	0.397 (0.704)							

χ^2 : Chi-square test, FE: Fisher Exact, p: p-value for the association between different categories, PPV: Positive predictive value, NPV: Negative predictive value.