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The abortion rate in trans-myometrial eggs retrieval is the same as in classical transvaginal retrieval

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Abstract. – OBJECTIVE: This study compares the miscarriage rate of pregnancies after trans-myometrial eggs retrieval to transvaginal eggs retrieval.

PATIENTS AND METHODS: In the period between January 2004 and December 2020, 13,323 egg retrievals were carried out. In 699 cases, the ovaries were unreachable. Alternative maneuvers were performed to solve this problem, but despite this, in 132 patients the technique of trans-myometrial sampling had to be used. 26 patients were excluded from the study, because of the inclusion criteria, and therefore two groups of 106 patients were selected, Group A and Group B (control).

RESULTS: In the comparison between the two groups, there were no statistically significant differences in abortion rates, pregnancy rates and complications after the technique.

CONCLUSIONS: This study shows that the abortion rate in trans-myometrial oocyte retrieval does not change when compared to classic retrieval, despite the sampling needle completely crossing the myometrium. Furthermore, the pregnancy rate and the complication rate do not appear to have worsened with this technique.

Key Words:
Abortion rate, Trans-myometrial follicular aspiration, Oocyte retrieval, Intra-cytoplasmic semen injection (ICSI).

Introduction

The egg retrieval procedure (Oocyte pick up - OPU) has long been performed by laparoscopy[1]. It involved the insertion of three instruments into the woman's abdomen by means of multiple small surgical incisions, while the needle was inserted into the lower right quadrant to aspire follicular fluid[2].

This laparoscopic approach has been progressively abandoned, due to the risks and complexity of the technique. Over time there has been a gradual replacement of laparoscopy with a transabdominal and subsequently transvaginal (TVUS)[3,4] ultrasound-guided egg retrieval (TAUS). In recent years, however, the TAUS has been progressively replaced by the TVUS, which is nowadays considered to be a direct and safer approach to egg retrieval as the needle does not pass through the abdomen. During the recovery of the TVUS oocytes, the transvaginal probe is used to evaluate the position of the ovaries and their anatomical relationships. The needle passes through the guide of the ultrasound probe and is carefully inserted into the follicles through the vaginal wall. This technique lasts about 10-15 minutes and requires a light sedation, with a short hospital stay after the procedure. Although this procedure is widely adopted due to the lower risks of surgical complications, some conditions of ovarian inaccessibility[5-8] slightly reduce its use. In these cases, TAUS could be preferred[9]. Several studies compared laparoscopy[10], TAUS and TVUS for oocyte retrieval with each other and provided evidence that TVUS is preferred, due to its shorter surgical time required and less invasive surgery. However, in less than 2% of TVUS patients, this procedure becomes impractical due to anatomical problems[11,12].

In this small number of cases, the position of the ovaries makes egg retrieval more difficult. In these cases, before moving on to other ways, once the bladder is empty, a series of maneuvers are carried out to attempt to perform again the egg retrieval *via* TVUS.

First these maneuvers consist in the abdominal manual pressure on the unreachable ovary[13]; if this maneuver is not successful then try to put the patient in the reverse Trendelenburg position. If, nevertheless, the ovary is still inaccessible try a pull-up by a forceps on the cervix, and if this

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maneuver is still ineffective, a transabdominal sampling or finally a transvaginal / trans-myometrial sampling has to be carried out.

So, in rare cases, during the OPU, it is necessary to introduce the needle through the myometrium to access the ovarian follicles. Similarly, this approach has also been applied to trans-myometrial embryo transfer (ET), in cases where the entry of the catheter through the external or internal uterine orifice had been impossible[14,15].

However, uterine contractions due to endometrial injury can translate into a lower pregnancy rate in women undergoing *in vitro* fertilization (IVF) protocols[13,16,17]. Data on the reproductive outcomes of this transfer route are still extremely poor.

Our study aims to verify whether transvaginal/trans-myometrial oocyte retrieval in women with inaccessible ovaries gives the same results in terms of outcome and risks compared to the classical method.

Patients and Methods

In this multicenter retrospective cohort study, female patients under the age of 44 were enrolled, candidates for intracytoplasmic sperm injection (ICSI) at "Momò Fertilife – Reproductive Medicine Center" in Bisceglie (Italy) and at "Ospedale Santa Maria" of Bari (Figure 1).

13,323 IVF cycles were performed in both infertility centers from January 2004 to December 2020. From this initial cohort, 699 women were found to have one or both ovaries inaccessible for transvaginal OPU.

These 699 patients were subjected to alternative maneuvers to make access to the ovaries possible, otherwise they were subjected to transabdominal sampling[18]. In all cases, the transabdominal access of the needle is followed using color Doppler to avoid injury to the blood vessels.

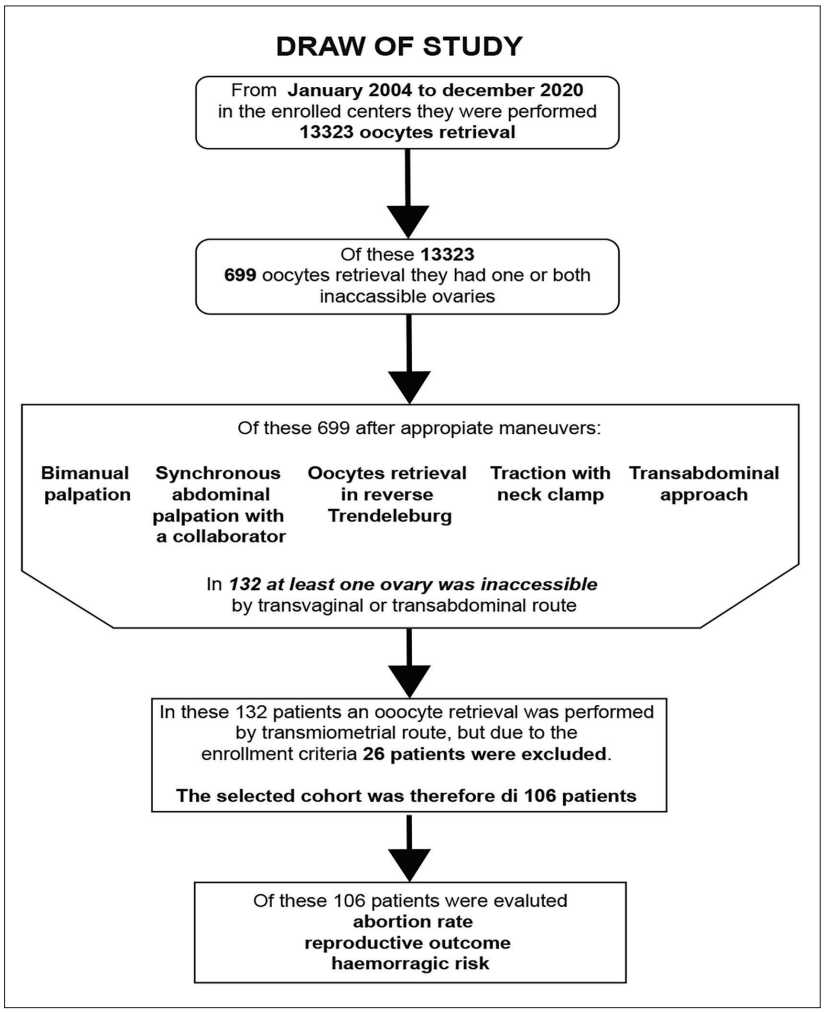

Figure 1. Drawing of the study.

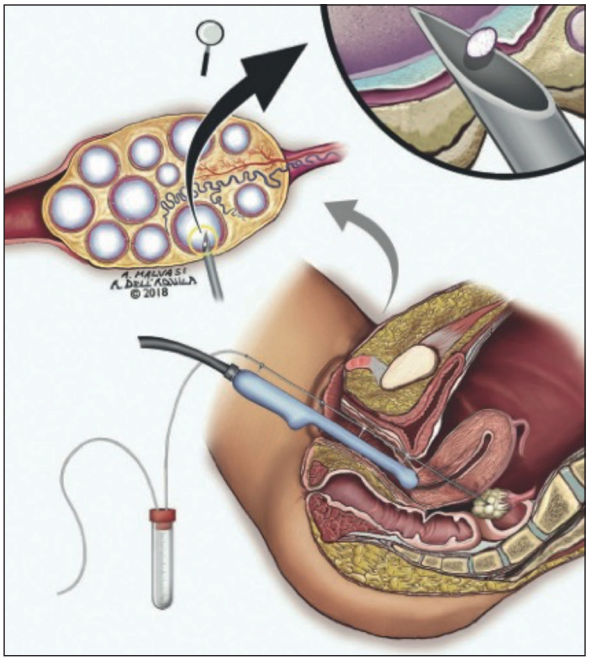

Figure 2. Schematic representation of trans-myometrial oocyte retrieval.

In 567 patients the maneuvers gave a favorable outcome, in 132 patients, despite the maneuvers used, it was necessary to resort to transvaginal/trans-myometrial sampling (Figure 2).

In this group of 132 patients, the following ones were adopted as exclusion criteria: couples with male partners who showed azoospermia and cryptozoospermia, couples with genetic pathologies, couples who underwent traditional fertilization and those who transferred on the fifth day. The last two exclusion criteria were included because the control sample would not have been consistent. Therefore, only 106 patients were included in the study.

The control group (called Group B) included 106 patients with classical transvaginal sampling, selected thanks to the MedITEX software, so that it was similar to the examined group by etiology, by age, by fertilization technique used (ICSI), by day of the transfer. The software, with a specific script, used a Random method of recruitment; thus, this made it possible to eliminate the selective bias related to the choice of samples from the control group (Table I).

Statistical Analysis

The study was configured as a retrospective observation of the type of case control study. The control group was randomly selected by the infertile couple management software (MedITEX), so that the parameters in Table I were as similar as possible. Data between groups were calculated as mean values (M) + DS while ANOVA was performed using the 8.0 Statistica version (StatSoft Italia Srl, Padua) and compared with the Student's *t*-test, using a p-value < 0.05 as a significance limit.

Later, for the evaluation of this retrospective cohort study, data regarding reproductive outcome and abortion risk were examined and later compared with the Student's *t*-test, using a p-value < 0.05 as the probability and significance limit.

Stimulation Procedure

All patients were stimulated with recombinant follicle stimulating hormone (FSH) (GONAL-f, Merck Serono, Germany), in a protocol with gonadotropin releasing hormone (GnRH) antagonists (Cetrotide, Merck Serono Germany), which involved the initiation of the antagonist administration in 7 days of stimulation, and finally induced final maturation with an alfa-choriogonad-

Table I. Comparison between Group A and the control Group B.

	Group A	Group B	*p*-value
Patient number	106	106	
Age	36.7 ± 0.9	36.7 ± 0.8	-
BMI	27 ± 0.8	27 ± 0.9	
Attempt number	2 ± 0.5	2 ± 0.4	-
Diagnosis			
Male factor	30	29	(NS)
Tubal factor	11	10	(NS)
Unexplained	20	19	(NS)
Oligo/anovulation	14	14	(NS)
Endometriosis	19	21	(NS)
Polycystic ovary	5	5	(NS)
Uterine	1	2	(NS)
Other	6	6	(NS)

otropin (Ovitrelle 250, Merck Serono Germany), when at least three follicles were larger than 18 mm.

Egg Retrieval Procedures

The patients were subjected to sedation for egg retrieval. The oocytes were collected by follicular puncture using a 17-gauge aspiration needle (COOK Medical, USA) connected to a guide on the transvaginal ultrasound device (Aloka, Japan; Toshiba, Japan; GE USA)[19]. When this was not possible, the maneuvers described in Table II were thus carried out.

In cases where even the maneuvers were not successful, transabdominal sampling was carried out; and finally, in cases in which transabdominal sampling had failed, transvaginal/trans-myometrial sampling was performed. The semen was collected after 3/4 days of sexual abstinence and prepared according to the method used in our center[20,21]. The cumulus-oocyte complexes were exposed to hyaluronidase solution (25 IU/ml) to remove the corona radiata and the clean oocytes were then inspected and evaluated under a stereomicroscope (Nikon SMZ 1500, Japan) to select those in metaphase II (MII). These eggs were then incubated in LGGF medium (Global Fertilization, Cooper Surgical, Trumbull, CT, USA) and injected 38-40 hours later.

Insemination Procedure

In both groups A and B, the ICSI procedure was performed at 37°C under an inverted microscope (Nikon Eclipse, TE 200), using a 400× magnification microinjection system. After the insemination, the fertilized oocytes were cultured in LGGG (Global, Cooper Surgical, Trumbull, CT, USA) medium for 3 days.

Transfer Procedure

On the third day of culture, all patients underwent transfer with fresh embryos. On average, two embryos were transferred. Patients having a high progesterone value on the hCG day were excluded from the study and therefore their embryos were frozen[22].

After 12 days, the beta hCG blood dosing was carried out to evaluate the presence or absence of pregnancy.

Results

Out of 13,323 egg retrievals carried out, only 699 were found to have inaccessible ovaries. In the end, only 132 resorted to transvaginal/trans-myometrial sampling equal to 0.99%.

Our first evaluation concerns the succession of the maneuvers performed, in those cases where transvaginal sampling was difficult or impossible (Table II).

In most cases, the problem was solved by a simple bimanual pressure on the abdomen or *via* a probe. This happened in 399 cases, equal to 57% of the sample. In 8 cases the reverse Trendelenburg position was adopted (1.1%). 20 patients benefitted from cervical traction with forceps (2.8%). In 140 (20%) patients, a transabdominal sampling was needed. Only in 132 (19.1%) patients all the maneuvers were ineffective and transabdominal sampling was impossible. Therefore, in these patients, the sampling was carried out transvaginally/trans-myometrially.

Among these 132 patients, 26 were excluded; thus, the final group on which the whole assessments were conducted was composed by 106 patients.

In order to assess the reproductive outcomes and the potential complications of this alternative procedure, Group A was compared to Group B control.

Both groups were compared in relation to different aspects, such as estradiol levels and number of follicles on the day of the hCG injection,

Table II. Sequence of maneuvers carried out for oocyte pick up in patients with inaccessible ovaries.

	1st maneuver	2nd maneuver	3rd maneuver	4th maneuver	5th maneuver
Bimanual examination or abdominal pressure or probe pressure	399 (57%)				
Trandelenburg position		8 (1.1%)			
Cervical clamp traction			20 (2.8%)		
Transabdominal pick-up				140 (20%)	
Transmyometrial pick-up					132* (19.1 %)

*106 enrolled because 26 did not meet the inclusion criteria.

Table III. Outcomes of IVF cycles in transvaginal/trans-myometrial oocyte retrievals (Group A) and controls (Group B).

	Group A	Group B	*p*-value
Estradiol level on day hCG	1728 ± 2214	1821 ± 147	0.14
Progesterone level on day hCG			
No. of follicles on day of hCG	11.2 ± 0.9	11.6 ± 0.5	0.10
No. of stimulation days	11.6 ± 0.5	11.5 ± 0.4	0.22
Total eggs	9 ± 1.8	9.5 ± 1.4	0.13
No. of mature eggs injected	5.0 ± 0.4	5.2 ± 0.3	0.09
No. of embryos	3 ± 0.6	3.2 ± 0.7	0.09
No. of embryo transferred	2 ± 0.3	2 ± 0.2	0.18
Ongoing pregnancy	33	34	0.09
Chemical pregnancy	10	11	0.11
Ectopic pregnancy	3	2	0.21
Abortion (week 12)	3	4	0.21

days of stimulation, number of oocytes retrieved and inseminated, number and grading of embryos transferred, number of cells and reproductive outcomes, including developmental pregnancy, ectopic and biochemical pregnancy.

Table III clearly shows that no significant differences were found between the parameters of the two groups ($p > 0.05$ in all cases). A further evaluation was made regarding the safety of transvaginal/trans-myometrial sampling, especially for the risk of causing peritoneal or endometrial bleeding. In Table IV the blood losses were evaluated by transvaginal ultrasound by the doppler color, after 2 hours, after 4 hours and after 24 hours. No cases of peritoneal or endometrial bleeding were reported after 24 hours (Table IV).

Discussion

The egg retrieval is generally performed by a follicular aspiration guided by TVUS[4], which has replaced conventional laparoscopic and transabdominal techniques[1-3], as studies have shown its safety, efficacy and simple use. Therefore, in this regard, several authors have tried to compare these different oocyte retrieval strategies, in terms of time required, invasiveness and safety[9].

Table IV. Bleeding risk by color-Doppler ultrasound on the posterior uterine wall in correspondence with the needle exit point in Group A.

Peritoneal or endometrial bleeding	Not	Yes
After 2 hours	86	20
After 4 hours	104	2
After 24 hours	92	0

Barton et al[13] reported in their study that TVUS follicular aspiration was a preferable procedure requiring less time and invasiveness, compared to TAUS, which according to some[12] could be considered a useful technique for retrieving oocytes in cases of inaccessible ovaries by means of the transvaginal approach.

In fact, literature data show that the TAUS approach can be successfully used mainly in women with radical hysterectomies, transposed ovaries[5,6], Müllerian agenesis[7,8,11], as well as in cases of increased body index mass (BMI)[23-25], for which the poor quality of the ultrasound image makes the ovaries unreachable.

However, despite the feasibility of this approach, recovering from TAUS often requires multiple punctures for each ovary with a strong impact on patients' comfort, as well as infectious risks[22], thus making the transvaginal approach preferable.

Therefore, in order to avoid inaccessible ovarian conditions recurring in approximately 2% of the women undergoing OPU, several management options are required, including urinary bladder emptying, manipulation of the vaginal scan probe with abdominal pressure, or a transvaginal/trans-myometrial sampling[26].

Generally, the passage of the needle through the myometrium, and in some cases through the endometrium, is not a conventional procedure as it could interfere with a subsequent embryonic implantation[14,27-29]. Anyway, the real effects of transvaginal/trans-myometrial recovery on reproductive outcomes and complications resulting from this practice are still poorly explored.

In their study, Davis et al[28]. showed that transvaginal/trans-myometrial recovery was performed in 1.7% of the samples, whereas in our study, carried out on an approximately double

population, we found the method used only in 0.99%. Furthermore, Davis demonstrated that this approach did not significantly influence the outcome of the pregnancy and we highlighted the same data too[28]. In his work, Davis points out the absence of possible bleeding risks, however without documenting data in this regard. In our study it is very clear that blood loss at 4 hours and 24 hours is absent, and this allows us to hypothesize that this method appears quite safe.

Our study aimed to verify if the data already present in the current literature, in particular the reproductive outcome and the potential complications deriving from the execution of a transvaginal/trans-myometrial sampling, agreed with those highlighted by us. Finally, we had the confirmation of the correspondence of what Davis had previously assessed.

Conclusions

The data presented in our study suggest that transvaginal/trans-myometrial sampling could be considered a safe approach in IVF programs, to be used only in the case of an inaccessible or unreachable ovary by alternative medical maneuvers. According to our data, this approach does not appear to be associated with a reduction in egg production or a decrease in the pregnancy rate and does not appear to be burdened with bleeding complications. Above all, it does not appear to be a risk factor for abortion.

It is necessary to carry out further studies with an adequate number of cases in order to confirm or not our findings. A multicenter study with a higher number of cases could definitively conclude the evaluations on the subject.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Informed Consent

Each patient signed a written informed consent consistent with standard criteria adopted in Italy and with some specific characteristics18. A specific note of informed consent reported the case of inaccessible ovaries and any surgical maneuvers other than the standard approach.

Ethics Committee Approval

The retrospective study was approved by the Local Ethics Committee of the MOMO' FERTILIFE with a specific note (code No. 0613 of 2004).

Authors' Contribution

G.M. Baldini: idea and drafting of the research protocol and writing paper; P. Totaro: data collection and patient selection; A. Malvasi: critical review of data; D. Baldini: critical review of the article and statistical evaluation.

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