



Transabdominal ultrasound–guided oocyte retrieval for oocyte cryopreservation using a vaginal probe: a comparison of applicability, effectiveness, and safety with conventional transvaginal approach

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Abstract

Purpose We aimed to compare the feasibility, effectiveness, and safety of transabdominal ultrasound–guided oocyte retrieval (TUGOR) using a vaginal probe and traditional vaginal approach in virgin patients undergoing oocyte cryopreservation.

Methods A total of 116 virgin patients who underwent transabdominal ultrasound–guided oocyte retrieval using a vaginal ultrasound probe and 33 patients matched for BMI, antral follicle count, age, day 3 FSH, estradiol, and AMH who underwent vaginal approach were enrolled. Mean number of total oocytes collected, mean number of cryopreserved MII oocytes, duration of the procedure, duration of stimulation, mean gonadotropin consumption, mature oocyte ratio, and a modified follicle–oocyte index were compared between the groups.

Results No statistical difference was found between the groups in mean number of follicles > 12 mm (4.62 ± 4.54 vs. 5.44 ± 4.52), mean number of oocytes collected (4.44 ± 4.14 vs. 5.33 ± 4.52), mean number of cryopreserved MII oocytes (4.01 ± 3.67 vs. 4.53 ± 4.13), mean duration of the procedure (12.4 ± 1.2 vs. 13.4 ± 1.6 min), mean days of stimulation (8.05 ± 1.91 vs. 8.35 ± 1.72 days), mean gonadotropin consumption (1507.9 ± 475.3 vs. 1571.74 ± 404.6 units), mature oocyte ratio (0.78 ± 0.24 vs. 0.82 ± 0.26), and modified follicle oocyte index (0.86 ± 0.63 vs. 0.84 ± 0.19). In the TUGOR group, superficial epigastric artery injury occurred in two patients and resolved spontaneously.

Conclusion Transabdominal oocyte retrieval using a vaginal ultrasound is a safe, effective, and feasible method of oocyte retrieval in some selected patient groups.

Keywords In vitro fertilization · Oocyte cryopreservation · Oocyte retrieval · Transabdominal follicular retrieval

Introduction

The gold standard approach to perform oocyte retrieval is transvaginal ultrasound (TVUS)–guided follicle aspiration due to shorter operation time, less invasiveness, and close proximity of the ovaries with the operating ultrasound probe. Before TVUS-guided follicular aspiration became a routine clinical practice, oocytes had been aspirated through direct laparoscopic guidance under general anesthesia or via transabdominal approach [1, 2].

Over the past two decades, due to increased success in cryopreservation technologies, the concept of fertility preservation has taken a great public interest worldwide. In recent years, an increasing number of young women, either diagnosed with decreased ovarian reserve or willing to postpone childbearing to late reproductive years, have been resorting to IVF clinics worldwide to freeze their oocytes as

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a panacea for ovarian aging. Among fertility preservation options, oocyte cryopreservation was accepted as a standard technique by American Society of Reproductive Medicine in 2012 following the reports of increased oocyte survival rates, especially using vitrification. However, a considerable proportion of these young women does not wish to undergo vaginal oocyte retrieval due to cultural precepts or religious constrictions.

In a limited number of previous case studies, transabdominal approach was suggested as a safe and effective procedure [3–5]. Transabdominal ultrasound-guided follicle aspiration was first described by Lenz et al. and then was implemented in some specific clinical circumstances such as in patients who previously underwent hysterectomy for a surrogate pregnancy, those with transposed ovaries and müllerian agenesis, and patients with distorted pelvic anatomy which prevents vaginal access [3–6]. In a previous study describing the technique, we demonstrated that transabdominal ultrasound-guided follicle retrieval using a vaginal probe is a feasible, effective, and safe method of oocyte retrieval for fertility preservation or in patients with inaccessible ovaries via transvaginal route undergoing IVF [7]. In this study, we aimed to compare the feasibility, effectiveness, and safety of transabdominal ultrasound-guided oocyte retrieval (TUGOR) using a vaginal probe and that of traditional vaginal ultrasound-guided oocyte retrieval in virgin patients undergoing oocyte freezing.

Material and methods

In a tertiary referral university IVF clinic and another private IVF clinic, a total of 116 patients who underwent TUGOR and 33 patients matched for BMI, antral follicle count, age, day 3 serum FSH, estradiol and AMH, who underwent conventional vaginal ultrasound-guided oocyte retrieval between January 2016 and June 2022, were enrolled in the study. In TUGOR group, all of the patients were virgin and refused to undergo transvaginal oocyte retrieval. All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards and Institutional Review Board approval was obtained before the patient enrollment.

This technique was first performed in a patient with multiple abdominal surgeries and inaccessible ovaries through the vaginal approach for oocyte retrieval, which resulted in a healthy live birth in 2011. Since then, TUGOR has been performed successfully where traditional transvaginal approach is not feasible due to acquired or congenital anatomic disorders in our university clinic. As previously described in detail, all patients underwent a standard ovarian stimulation

protocol with individualized gonadotropin dosing ranged between 150 and 225 IU/day according to ovarian reserve parameters, previous ovarian response where existed, BMI, and age [7]. A combination of recombinant FSH (Gonal F, Merck-Serono) and highly purified human menopausal gonadotropins (Menopur, Ferring Pharmaceuticals) was used for controlled ovarian stimulation in all patients. Gonadotropins were started on day 2 or 3 of the menstrual cycle and continued until hCG trigger day. Random start controlled ovarian stimulation was performed if patients who applied in an urgent condition as initially described by us in 2011 [8]. A multiple dose flexible gonadotropin releasing hormone (GnRH) antagonist protocol (Cetrotide 0.25 µg/day s.c., Merck-Serono) was initiated when the leading follicle was > 13–14 mm and/or serum estradiol concentration was > 350–400 pg/mL. Follicular development was monitored using a transabdominal ultrasound probe, serial serum estradiol, luteinizing hormone (LH), and progesterone measurements. Short-term indomethacin was used if LH levels were > 10 IU/mL during ovarian stimulation. Oocyte retrieval was performed under sedation anesthesia with intravenous propofol administration. Oocyte retrieval was performed 35–36 h after dual trigger method described by us before when ≥ 1 –2 follicles reached ≥ 18 mm in diameter with a double lumen oocyte retrieval needle (Cook Medical, Australia) [9]. Oocyte retrieval was performed with a 17-gauge double lumen oocyte aspiration needle (Cook Medical, Australia).

Main outcome measures compared between the groups include indications for oocyte retrieval, total number of oocytes collected, total number and percentage of mature oocytes, duration of the procedure, complication rates, mature oocyte ratio, and a modified follicle oocyte index ratio. Mature oocyte ratio is calculated by dividing the total number of mature oocytes by total number of collected oocytes, and mFOI is calculated by dividing the total number of collected oocytes by total number of antral follicles ≥ 12 mm at trigger day. Independent variables were compared with Mann–Whitney *U* test between the groups, and $p < 0.05$ was considered statistically significant. SPSS Statistics package version 21.0 (Chicago, USA) was used for all statistical analysis.

Description of the technique — transabdominal ultrasound-guided follicular aspiration for oocyte retrieval

As described earlier in an algorithmic approach, a standard transvaginal ultrasound probe (4–8 MHz vaginal probe; Shimadzu, Japan, and Logic P5, General Electric, USA) was used to perform oocyte retrieval in all procedures [7]. Following copious pelvic cleansing using sterile saline, only sterile saline solution was applied

on the respective pelvic area during the whole TUGOR procedure to provide ultrasound beam conductivity. We opted to use vaginal probe due to its pointed tip with less surface area compared to abdominal convex probe, which allows the physician to implement a targeted pinpoint pressure on the interest area during oocyte retrieval. The transvaginal probe was placed on the ipsilateral ovary to prevent bowel, bladder, and vessel injuries by applying continuous pressure at point of interest area (Fig. 1). In contrast to abdominal ultrasound examination, the bladder was emptied in all patients before the procedure to ensure the proximity of ovaries with the applying vaginal probe. At this stage, if bowel segments, big vessels, or bladder are visualized within the area of examination just under the probe, the continuous applying pressure using the probe moves all risky anatomical structures away, ensuring the proximity between the follicles and the needle (Fig. 2). Color Doppler was rarely used to identify blood vessels if they were not easily recognizable on grayscale ultrasound examinations.

During TUGOR and conventional transvaginal approach, a 17-gauge double lumen oocyte aspiration needle (Cook Medical, Australia) was used for all oocyte retrieval procedures. A suction pressure of 150–180 mmHg was utilized (Labotec precise aspiration pump, Germany). Oocytes and embryos were frozen using a pre-prepared homemade vitrification media, including dimethyl sulfoxide (DMSO), ethylene glycol, and sucrose, and placed on a special cryodevice (EasyVit, i-reprotech, Turkey) before plunged in liquid nitrogen. Mature oocytes are metaphase II oocytes that extrude the first polar body and fertilization defined as the occurrence of two pronuclei. All MI oocytes were kept in overnight incubation and frozen as MII, only if first polar body was extruded.



Fig. 2 Targeted downward pressure application using vaginal ultrasound probe onto the ipsilateral ovary with an empty bladder

Results

In the current study, a total of 149 patients who applied for fertility preservation were enrolled in the study. The indications for TUGOR and vaginal approach between patients undergoing TUGOR and conventional vaginal approach are as follows, respectively: virgin women with decreased ovarian reserve who opted oocyte cryopreservation ($n = 93$, 80.1% vs. $n = 28$, 84.8%), breast cancer ($n = 10$, 8.6% vs. $n = 2$, 6.0%), lymphoma ($n = 1$, 0.86% vs. $n = 1$, 3.0%) endometrioma ($n = 7.6.0\%$ vs. $n = 1$, 3.0%), immature teratoma of the ovary ($n = 1$, 0.86% vs. $n = 1.3.0\%$), borderline ovarian tumor ($n = 3$. 2.5% in the TUGOR group), and retroperitoneal sarcoma ($n = 1$, 0.9% in the TUGOR group) (Table 1).

Fig. 1 The technique demonstrating single operator transabdominal ultrasound-guided follicular aspiration for oocyte retrieval



Table 1 Details of indications for oocyte freezing in the TUGOR and conventional vaginal oocyte retrieval groups

Indications	TUGOR Group	Conventional vaginal group
Virgin women with decreased ovarian reserve	93 (80.1%)	28, 84.8%
Breast cancer	10 (8.6%)	2 (6.0%)
Lymphoma	1 (0.86%)	1 (3.0%)
Endometrioma	7 (6.0%)	1 (3.0%)
Immature teratoma of the ovary	1 (0.86%)	1 (3.0%)
Borderline ovarian tumor	3 (2.5)	-
Retroperitoneal sarcoma	1 (0.86%)	-
Total	116	33

* All values are given as number and % in parenthesis

Table 2 Baseline parameters in the TUGOR and conventional vaginal oocyte retrieval groups

Parameter	TUGOR group	Conventional vaginal group	<i>p</i>
Age, years	32.9 ± 7.98	35.18 ± 8.75	> 0.05
BMI	27.8 ± 3.1	28.4 ± 2.9	> 0.05
AMH, ng/ml	0.82 ± 0.9	0.78 ± 0.71	> 0.05
FSH, IU/l	8.06 ± 2.44	9.3 ± 3.68	> 0.05
Estradiol, pg/ml	61.98 ± 26.59	68.35 ± 30.35	> 0.05
LH, IU/l	6.75 ± 8.26	5.65 ± 1.84	> 0.05
Progesterone, ng/ml	0.58 ± 0.48	0.69 ± 0.77	> 0.05

All variables are given as mean ± SD

BMI body mass index, *AMH* anti-Müllerian hormone, *FSH* follicle stimulating hormone, *LH* lutinizing hormone

There was no difference in baseline patient characteristics, including age, serum AMH, FSH, and estradiol levels between the groups (Table 2). Moreover, no difference was found in terms of mean days of stimulation (8.05 ± 1.91 days

vs. 8.35 ± 1.72 days) and total gonadotropin consumption (1507.91 ± 475.33 units vs. 1571.74 ± 404.68 units), between the TUGOR and conventional vaginal approach groups, respectively.

No statistical difference was found between the groups in terms of mean number of follicles > 12 mm (4.62 ± 4.54 total vs. 5.44 ± 4.52 , $p = 0.253$), oocytes collected (4.44 ± 4.14 vs. 5.33 ± 4.52 ; $p = 0.081$), mean number of cryopreserved MII oocytes (4.01 ± 3.67 vs. 4.53 ± 4.13 , $p = 0.161$), mean duration of the procedure (12.4 ± 1.2 min vs. 13.4 ± 1.6 min, $p > 0.05$), mature oocyte ratio (0.78 ± 0.24 vs. 0.82 ± 0.26 ; $p > 0.05$), and FOR (0.86 ± 0.63 vs. 0.84 ± 0.19 , $p = 0.097$) (Table 3). In TUGOR group, superficial epigastric artery injury occurred in two patients and resolved spontaneously. Notably, TUGOR was safely performed in 4 patients with previous midline incision for ovarian surgery without any complication (Fig. 3). Random start ovarian stimulation was performed in four patients with breast cancer in the TUGOR group. Since previous studies demonstrated higher cumulative gonadotropin dose and longer stimulation duration in random start protocols, these four patients were excluded from the analysis in respective parameters [10].

Discussion

In this current retrospective study, we demonstrated that transabdominal ultrasound-guided oocyte retrieval using a vaginal probe is comparable to conventional transvaginal technique in terms of effectiveness and safety in patients undergoing IVF for oocyte cryopreservation in matched patient groups.

Fertility rates declined sharply over the 50 years in modern industrialized world mainly owing to fewer deaths in childhood, greater access to contraception, and an increased number of women pursuing education and seeking to

Table 3 IVF outcome data between the TUGOR and conventional vaginal oocyte retrieval groups

Parameter	TUGOR group	Conventional vaginal group	<i>p</i>
Number of follicles, <i>n</i>	4.62 ± 4.54	5.44 ± 4.4	> 0.05
Number of oocytes collected, <i>n</i>	4.44 ± 4.14	5.33 ± 4.52	> 0.05
Number of frozen MII oocytes, <i>n</i>	4.01 ± 3.67	4.53 ± 4.13	> 0.05
Duration of the procedure, min	12.4 ± 1.2	13.4 ± 1.6 min	> 0.05
Mature oocyte ratio*, %	0.78 ± 0.24	0.82 ± 0.26	> 0.05
Follicle output ratio#, %	0.86 ± 0.63	0.84 ± 0.19	> 0.05
Duration of stimulation, days	8.05 ± 1.91	8.35 ± 1.72	> 0.05
Cumulative gonadotropin consumption, units	1507.91 ± 475.33	1571.74 ± 404.68	> 0.05

All variables are given as mean ± SD

*Mature oocyte ratio is calculated by dividing total number of mature oocytes with total number of collected oocytes

#Follicle output ratio is calculated by dividing total number of follicles at trigger day ≥ 12 mm by total number of collected oocytes



Fig. 3 Needle insertion point in a patient previously underwent ovarian surgery using a midline incision

establish their careers before childbearing. In addition, according to the United Nations estimates, the mean age of first childbirth has increased over the age of 30 s in many developed countries [11]. For most parts of the world, having children is enormously essential, and in most cultures, being childless is considered an undesired social role. It was clearly demonstrated that involuntary childlessness has strong psychological consequences [12, 13]. Among the psychological and psychosomatic consequences that dramatically affect patients are distress, raised depression and anxiety levels, lowered self-esteem, blame and guilt, somatic complaints, and reduced sexual interest [12]. In this context, the concept of fertility preservation has been gaining increased interest for affected women either undergoing gonadotoxic treatments, gonadal surgery, or facing the risk of diminished ovarian reserve in the early reproductive ages.

Owing to significant progress and innovations in cryopreservation technology and following successful reports of healthy live births using vitrification, in the year 2012, ASRM declared an opinion surrounding oocyte freezing for fertility preservation and announced that it is no longer experimental. In a literature survey, medical reasons for oocyte cryopreservation included primary ovarian insufficiency, traumatic ovarian injury, planned female to male gender transition, and age-associated fertility loss or premature ovarian failure [14]. In some specific areas of the world, most of the single women are either not eligible due to young ages or unwilling to undergo vaginal oocyte retrieval due to cultural or religious reasons. In our study population, the prevailing indication was social oocyte

freezing due to diminished ovarian reserve, followed by patients diagnosed with breast cancer and lymphoma scheduled to undergo gonadotoxic chemotherapy.

Transabdominal oocyte retrieval using ultrasound was first described by Lenz et al. in 1981 [4]. Following this initial report, the technique was practiced successfully in some small patient groups using an abdominal probe [3]. In 2011, Barton et al. investigated the efficacy of ultrasound-guided transabdominal follicular aspiration in 69 patients with inaccessible ovaries and compared with another 69 transvaginal ultrasound-guided follicular aspiration controls matched by age, follicle number, and procedure year [15]. The total number of oocytes retrieved was less in the transabdominal group than in the transvaginal group (11.9 vs. 14.1, $p = 0.008$). However, there was no statistically significant difference in the number of mature oocytes (9.2 vs. 7.3, $p = 0.14$). Of note, in their study, a transabdominal vector 4–1 MHz probe was used for oocyte retrievals.

Transabdominal ultrasound-guided oocyte retrieval was successfully applied in patients with Müllerian anomalies. Damario reported a successful oocyte retrieval using a vaginal probe for the purpose of transabdominal oocyte retrieval in a patient with Müllerian agenesis [16]. In that report, 6 oocytes were retrieved, and two cleavage-stage embryos were transferred to the gestational carrier resulting in a twin pregnancy. Pereira et al. implemented a combined transvaginal and transabdominal oocyte retrieval approach in a patient with an ectopic ovary and unicornuate uterus. Transvaginal retrieval of oocytes from the right ovary was not possible due the anatomic location of the ovary, intervening blood vessels, and limited mobility of the ovary. They successfully retrieved 15 oocytes in 2 attempts, four blastocysts were frozen, and a live birth occurred following a single frozen thawed embryo transfer [17]. Selvaraj et al. reported successful percutaneous oocyte retrieval in a patient with a transposed right ovary who had previously underwent radical hysterectomy. Following retrieval of 5 oocytes, sequential transfer of 3 embryos in a surrogate carrier resulted in a healthy live birth. In 2018, Baldini et al. first compared the safety and effectiveness of transvaginal and transabdominal ultrasound-guided approaches using the same vaginal ultrasound probe in the same 21 patients [18]. There was no difference in terms of total number of oocytes retrieved, MII oocytes, and fertilization rates. In their series, they underlined the importance of single entry, and they asserted that due to the lesser elasticity of the abdominal wall, the transabdominal follicular aspiration may require multiple ovarian punctures through different abdominal wall entries, which may cause patient pain and increase the risk of injury to the viscera. Importantly, no intraoperative or postoperative complication was reported in any of the approaches applied.

One of the strengths of the current study may be that it has the greatest enrolled patient numbers in the literature for abdominal oocyte retrieval. No statistical difference was found between the groups in terms of mean number of total oocytes collected, mean number of cryopreserved MII oocytes, and mean duration of the procedure. In a further analysis, we also demonstrated that “mature oocyte ratio,” which is defined as the ratio of total number of mature oocytes/total number of collected oocytes, and “mFOI index,” which is defined as the ratio of total number of collected oocytes/total number of follicles at trigger day ≥ 12 mm, were also similar between the groups. Regarding complication rates, superficial epigastric artery injury occurred in two patients in the TUGOR group and resolved spontaneously, both of which occurred in the initial cases. Following these two experiences, by mapping epigastric arteries with the aid of color Doppler imaging and avoiding the needle insertion from the 3–4-cm distance of the midline where epigastric arteries anatomically lie, we did not experience such a complication in the following cases. Some authors asserted that transabdominal oocyte retrieval may be easier in patients having BMI > 40 kg/m² with inaccessible ovaries via vaginal route; however, we observed that high BMI might make the procedure more difficult due to increased subcutaneous fat tissue [15]. We did not experience any technical difficulty in patients with ovarian pathologies including borderline ovarian tumors, ovarian teratomas, and endometriomas. Notably, we observed the enlarged ovary made the procedure much easier.

In the initial reports, where transabdominal oocyte retrieval was performed using abdominal ultrasound probe with a full bladder yielding a sonolucent pelvic cavity, the authors reported transient hematuria in four patients. In their series, the authors inserted the oocyte pick-up needle through the bladder into the follicles [4]. In our technique since the bladder was empty, it might have been possible to not puncture the bladder and we did not encounter such a complication. In addition, we could have safely performed TUGOR in 4 patients with previous abdominal surgery using midline incision, demonstrating its safety record in experienced hands. By using a vaginal probe, we exploited the advantage of implementing continuous downward pressure during the whole procedure, which moves all risky anatomical structures away, mainly the bladder and intestines, and ensures proximity of index ovary and the aspiration needle. Another important technical benefit of using vaginal probe is that since it is attached with a biopsy needle, it is able to keep the needle stable during the procedure.

The main limitation of the study may be that the number of enrolled patients was lower compared to TUGOR group, 33 vs. 116, respectively. This difference primarily stems from the finding that the prevailing indication was social freezing owing to decreased ovarian reserve in

virgin women. In Turkey, the legislative regulations surrounding oocyte cryopreservation only allow freezing of oocytes in patients facing the risk of decreased ovarian reserve due to chemo-radiotherapy, those undergoing ovarian surgery, or patients with diminished ovarian reserve. These strict limitations clearly decrease the success rate of “social oocyte freezing.” In our series, 3 patients in the TUGOR group and 2 in the conventional group returned to use frozen oocytes, ending up with 2 healthy live births in TUGOR group and 1 healthy live birth in the conventional vaginal group. Although pregnancy rates fall out of the scope of this study, and the use rate of frozen oocytes is $< 10\%$ in our series, it is of note to mention here that a 39-year-old patient who underwent TUGOR that resulted in retrieval and freezing of one metaphase II oocyte conceived and delivered a healthy infant with this frozen-thawed oocyte when she was 44 years old. Moreover, in the year 2011, we successfully applied this technique in a patient for oocyte retrieval with inaccessible ovaries through the vaginal approach due to multiple abdominal surgeries ending up with a healthy live birth. Moreover, the positive psychosomatic impact of counseling the patients facing the risk of ovarian failure regarding the established fertility preservation technologies should never be underestimated. In this context, in a survey study, we previously demonstrated that offering a fertility preservation option to reproductive-aged cancer patients has a tremendous positive impact on psychological well-being and therapy adherence [19].

In some certain world areas where virginity is still of outmost cultural, social, and religious importance, and being childless is acknowledged as an undesired social status, preserving fertility by means of oocyte freezing clearly has an enormous positive impact on the quality of life of the affected women. Besides existence evidence demonstrating low use rates in patients undergoing social oocyte freezing, and success rates decrease with age and with diminished ovarian reserve, autologous oocyte cryopreservation is the only valid option in countries where oocyte donation is still not allowed by the legislative authority. TUGOR using a vaginal probe may be a safe and effective option for this specific group of patients. The abdominal approach avoids the psychological burden and the risks of inadvertent hymenectomy for virginal patients at the time of the vaginal procedure. Furthermore, TUGOR may also be an acceptable alternative method for female to male transgenders or any gender expansive wishing to avoid any vaginal procedure.

Author contribution Meltem Sönmezer and Çağrı Gülümser conceived and designed the study. Yavuz Emre Şükür, Cem Atabekoğlu, Murat Sönmezer, and Sinan Özkavukçu performed the experiments. Koray Görkem Saçını and Bulut Varlı gathered and analyzed the data. Meltem Sönmezer, Koray Görkem Saçını, and Murat Sönmezer wrote

the draft and revised the manuscript. All authors read and approved the final manuscript.

Data availability Requests for material should be made to the corresponding authors.

Code availability Not applicable.

Declarations

Ethics approval and consent to participate All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards, and Institutional Review Board of Ankara University (Ankara, Turkey) approval was obtained before the patient enrollment. A written informed consent for participating in this trial was obtained from all patients.

Consent for publication All authors have provided consent for publication. Patients signed informed consent regarding publishing their data.

Conflict of interest The authors declare no competing interests.

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