—Mini Review—

Evidence-based Oocyte Aspiration and Embryo Transfer

Koichi Kyono[1]

[1]*Ladies Clinic Kyono, Kyono Reproduction Research Center, 3-8-6 Omiya, Furukawa City, Miyagi 989-6221, Japan*

Abstract: *When the first successful delivery following in vitro fertilization and embryo transfer was reported in 1978, oocyte aspiration was performed laparoscopically under general anesthesia. Since 1985, almost all IVF centers have collected oocytes using transvaginal ultrasound-directed methods, since these are the easiest, most accurate and most acceptable methods to patients. Color Doppler ultrasonography is recommended to decrease blood loss during oocyte aspiration. Embryos are gently, slowly and transcervically expelled into the uterine cavity, with the patient in a lithotomy position. This basic method has remained unchanged since the first descriptions. Relatively important factors for successful embryo transfer include removal of hydrosalpinges, absence of blood or mucus on catheter, catheter type, avoidance of fundus contact, avoiding tenaculum, removal of all mucus, ultrasonography of cavity before puncture, leaving the catheter in place for 1 min, 30-min bed rest, trial transfer, ultrasonographic monitoring and antiprostaglandin administration to prevent uterine contractions.*

Key words: *Oocyte aspiration, Embryo transfer, Ultrasound, Aspiration needle, Evidence*

Initially oocytes were obtained from ovaries or pieces of ovaries by laparotomy [1]. As early as 1968, Steptoe devised a method of aspirating oocytes laparoscopically from human preovulatory follicles [2]. In 1976, a pregnancy was reported following in vitro fertilization (IVF) using oocytes from laparoscopic oocyte recovery, although this first-ever IVF pregnancy was subsequently found to be ectopic [3]. A further 2 years passed before a successful live birth following IVF was achieved [4].

Wikland and colleagues first suggested the potential of vaginal ultrasonography for oocyte recovery in 1985, and at the same time described methods for guiding the collecting needle alongside the transducer to achieve very accurate puncture of follicles [5]. Now, almost every IVF unit collects oocytes using transvaginal ultrasound-directed methods, since these are the easiest, most accurate and most acceptable methods to patients.

The basic method of embryo transfer (ET) in humans has remained basically unchanged since the first description by Edwards *et al.* nearly 20 years ago [4]. Meldrum *et al.* were among the first investigators to suggest that meticulous ET technique is central to successful IVF [6].

The present paper reviews published data on techniques for oocyte aspiration and ET and includes perspectives based on the experiences of the author in an attempt to determine optimal oocyte retrieval and ET techniques.

Oocyte aspiration

Timing of oocyte retrieval

An ovarian stimulation protocol using gonadotropin releasing hormone analogues (agonist or antagonist) and follicle stimulating hormone (GnRHa/FSH) is used almost universally in assisted reproductive technology (ART).

When ≥3 follicles reach a diameter of >17 mm and serum estradiol concentrations are approximately 200-300 pg/ml, final oocyte maturation is initiated by intramuscular injection of human chorionic gonadotropin (hCG; 3,300–10,000 IU). Oocyte retrieval is scheduled to precede ovulation, about 34–36 h after hCG injection [7].

Anesthesia

Light anesthesia is most acceptable, as the patient is

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e-mail: info@ivf-kyono.or.jp

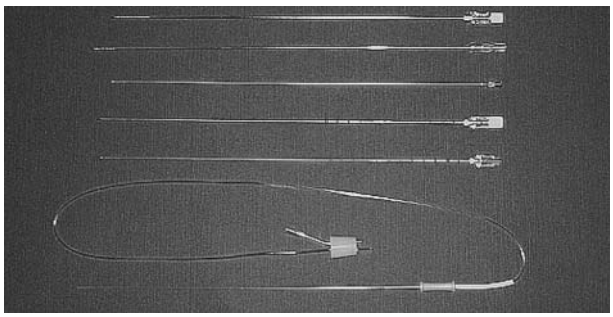

Fig. 1. Aspiration needles. From top to bottom: 1. NM-1831 N 18GX 31cm, Naka Medical Co., 2. ON1733 17Gx 33cm, Smith Medical Japan Co., 3. FS-N1932-PZ, Kitazato Supply Co., 4. FS-N1932-R, Kitazato Supply Co., 5. FS-N-1932-P, Kitazato Supply Co., 6. FS-NK2130-K, Kitazato Supply Co.

asleep, has no memory of the procedure, wakes within 5 min of completion of oocyte collection, and is typically able to return home within 1–2 h [6].

Materials checklist for transvaginal oocyte retrieval

A. Aspiration needle (Fig. 1)

The aspirating needle (length, 30–35 cm) can be either single (17–21 gauge) or double lumen (14–17 gauge) [7]. A single lumen needle is useful due to its small diameter and low risk of complications. When multiple follicle flushes are needed (for example, in natural cycle IVF), a single lumen needle directly connected to a syringe can be used for manual flushing. In the experience of the author, flushing is unnecessary in most cases.

B. Suction device

i) Regulated aspiration pumps (Table 1)

For oocyte collection, vacuum pressure in the collection system is usually <20 kPa (150 mmHg) [7, 8]. A suction pressure of 20 kPa (150 mmHg) is estimated to require ≤5 s for the above system to stabilize to the selected vacuum. Suction pressure differs according to the length and diameter of the needle, follicle size, syringe volume by manual or regulated aspiration pumps [9]. For example, a 35-cm long 18 G needle (inner diameter, 0.85 mm) requires an aspiration pressure of 180–250 mmHg.

ii) Manual suction by syringe

Cohen *et al.* compared the effects of manual and mechanical suction on zonal damage [10]. Using suction apparatus may be better than manual suction. However, manual suction by syringe is currently very simple, easy and low cost, and is thus widely used for oocyte aspiration in Japan. Use of the minimum pressure is recommended to avoid damage to the oocyte.

C. Transvaginal ultrasonograph (B-mode vs Color Doppler)

At the Ladies Clinic Kyono (LCK), an MEU-1585 Luketron ultrasonograph (Mochida, Japan) with a 7.5-MHz transvaginal probe is used for all transvaginal oocyte retrievals. Color Doppler ultrasonography has recently been trialed at LCK for oocyte aspiration (Fig. 2). Color Doppler ultrasonography is useful to decrease blood loss during oocyte aspiration. The major problem in color Doppler ultrasonography is that motion artifacts obscure visualization of the needle tip during follicle puncture. The tip of the needle can be seen in B-mode, allowing simultaneous confirmation of vessel positions on color Doppler ultrasonography in some ultrasound machines.

Ultrasound-guided retrieval techniques

Most materials are preheated to 37°C in a warming box. At LCK, an aspiration needle-connected syringe is used for all transvaginal oocyte retrievals. The ovaries are localized and lined up using the indicator line on the imaging screen. The follicle closest to the probe is entered using a short, controlled stabbing motion. A more progressive drilling motion is suggested for follicles localized on the posterior side of the ovary in the vicinity of major pelvic blood vessels or bowel loops.

The needle tip is kept at the center of the follicles while the surrounding follicle wall collapses. The operator should ensure that the follicle is completely emptied. Once the follicle is aspirated, the fluid is immediately sent to the embryologist. To reduce the

Table 1. Vacuum pumps

Company	Type	Max. aspiration pressure (mmHg)
Kitazato Supply Corporation	KAS-3000	–650
Cook Australia	K-MAR-5100	–300
Washiesu Surgical Instruments	ECV-01	–300
Atom	VP-400	–750
Craft	R29.654	–100

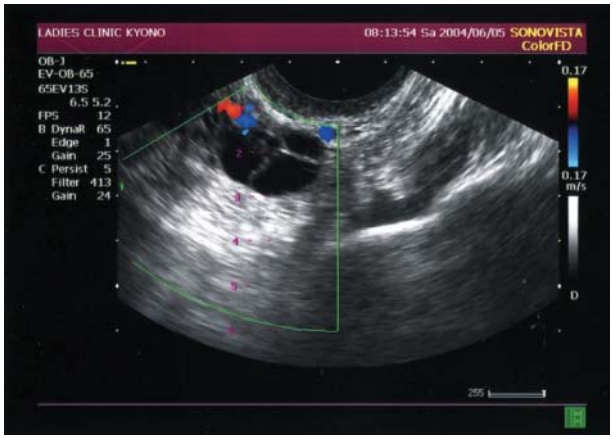

Fig. 2. Color Doppler ultrasonography. Vaginal vessels and follicles.

Table 2. B-mode ultrasonographs

Company	Type
Mochida medical system	MODEL MEU-1585
Aloka Corporation	ProSound SSD-6500
Toshiba Medical System Corporation	NEMIO
Hitachi Medico Corporation	EUB-6500
GE Yokokawa Medical System	LOGIQ 100 pro

Table 3. Color Doppler ultrasonographs

Company	Type
Mochida Medical System	MODEL MEU-1588
Aloka Corporation	ProSound SSD-3500
Toshiba Medical System Corporation	NEMIO SSA-550A
Hitachi Medico Corporation	EUB-5500
GE Yokokawa Medical System	LOGIQ3 J-version

adverse effect of temperature fluctuations on oocytes, the distance between the patient and embryology laboratory should be minimal.

Complications

A. Intra- or retro-peritoneal bleeding

Dessole *et al.* estimated blood loss within 24 h after oocyte retrieval as about 230 ml [11]. Frequency of severe intra- or retro-peritoneal bleeding is reportedly 0–1.3%. Intraperitoneal bleeding tends to be severe with acute hemodynamic deterioration, whereas retroperitoneal bleeding usually displays a later and more indolent presentation. Laparoscopic drainage and hemostasis or laparotomy may be required in severe cases [12].

B. Vaginal bleeding

Bleeding from the vaginal vault is a common consequence of oocyte aspiration, occurring in 1.4–18.4% of procedures [13]. In most cases, vaginal bleeding as a result of oocyte retrieval resolves spontaneously at the end of the procedure. The bleeding site needs to be identified by vaginal exploration using a large speculum, followed by the application of pressure vaginal packing using a gauze roll.

C. Pelvic inflammatory disease

Pelvic inflammatory disease is an infrequent complication, occurring in 0.2–0.5% of cycles. Patients need treatment with intravenous antibiotics and may require culdocentesis for adequate pelvic abscess drainage or adnexectomy [14].

Embryo transfer

ET represents the final and most crucial step in IVF. Successful implantation after ET depends on various factors, including embryo quality, endometrial receptivity, and the ET technique itself.

The present review examines only the techniques for ET.

Removal of hydrosalpinges before treatment

Hydrosalpinx suction or removal of hydrosalpinges before ET is recommended, as the presence of hydrosalpinges has been found to exert a negative influence on embryo implantation due to possible deleterious effects on endometrial receptivity [14–17].

Absence of blood or mucus on catheter

Reports generally agree that bleeding and cervical trauma at the time of ET result in a diminished chance of embryo implantation [18].

Type of catheter (Fig. 3)

Marconi *et al.* used microhysteroscopy to visualize possible lesions on the endocervix and endometrium made by various catheters (Tomcat catheter, Frydman's catheter, Frydman's set, Wallace's catheter) commonly used for ET [19]. The Wallace catheter appears to result in the least trauma to the endometrium. An ultrasonographic study evaluating intrauterine insemination found endometrial disruption in 50% of women in whom a Tomcat catheter was used,

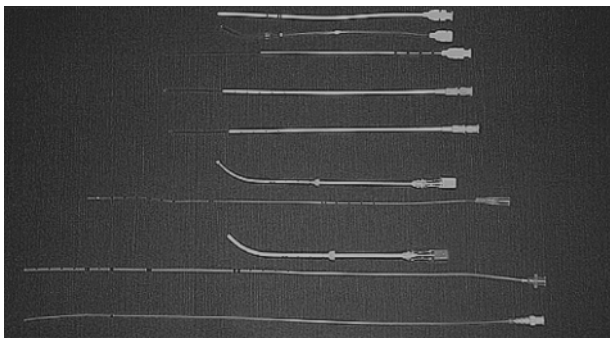

Fig. 3. Embryo transfer catheters. From top to bottom: 1 K-PAI-1000, Cook Australia, 2 K-JETS-7019-SIVF (guiding catheter), Cook Australia, 3 K-JETS-7019-SIVF (transfer catheter), Cook Australia, 4. 2316, Smith Medical Japan Co., 5. 1816N, Smith Medical Japan Co., 6. 7. FS-ET319GR232S, Kitazato Supply Co., 8. FS-ET6-ST17, Kitazato Supply Co., 9. FS-ET640S, Kitazato Supply Co., 10. 0197102, Fuji System Co.

compared with only 12.5% with use of the Wallace catheter [20]. Other studies also found that the Wallace catheter appears to result in the least trauma to the endometrium [21, 22].

Ultrasound-directed embryo transfer (avoiding contact with the fundus)

The ET catheter may be inserted either blindly by "clinical touch", or under ultrasonographic guidance.

Prapas *et al.* [23] described a significantly improved pregnancy rate in 61 ultrasound-directed ETs when compared with 71 blind insertions (36.1% vs. 22.6%, respectively). Coroleu *et al.* [24] conducted a prospective randomized trial of 362 patients comparing ultrasonography with clinical touch, and reported a significantly increased pregnancy rate using ultrasonographic techniques (50% vs. 33.7%, respectively).

Dummy transfer before treatment

Dummy transfer before treatment allows the physician to measure depth and direction of the uterine cavity, and this can prove useful given the wide variability in cervical and uterine anatomy. Routine performance of trial transfer 1–2 months before the IVF cycle appears recommendable. Cervical stenosis or acute angulation between the cervix and uterus can limit uterine access and make ET difficult. Abusheikha *et al.* [25] found that cervical dilatation before initiation of the treatment cycle resulted in easier transfer and improved pregnancy rates. If cervical stenosis or angulation persists at the time of transfer, the Towako method (transmyometrial transfer) can be used [26].

Bed rest after transfer

Sharif *et al.* demonstrated a favorable pregnancy rate with no bed rest following ET [27]. Botta and Grudzinskas found that prolonged bed rest (24 h) was not associated with better outcomes compared to a 20-min rest period after ET [28]. Woolcott and Stanger demonstrated no movement of the embryo-associated air bubble on standing shortly after ET, as assessed on ultrasonography [29]. Baba *et al.* evaluated 60 ETs resulting in 22 pregnancies and 32 gestational sacs, finding that in pregnancies achieved through ET, approximately 80% of embryos implant in the areas to which they are initially transferred, while approximately 20% implant in other areas [30].

Loading the catheter

Meldrum *et al.* reported increases in pregnancy and implantation rates after reducing the amount of air and total transfer volume in the catheter [6]. At the LCK Center for Reproductive Medicine, approximately 10 μl of medium containing the embryos and 5 μl of air lock at the tip is used in a Wallace catheter attached to 1-mL airtight syringe. Replacing protein in the transfer medium with glycosaminoglycan hyaluronan has been shown to significantly improve outcomes in a mouse model [31].

Placement of catheter tip

Waterstone *et al.* [32] reported a 46% pregnancy rate (45/98 patients) if the fundus was not touched during ET, compared to 24% (33/137 patients) if the fundus was touched. Yovich *et al.* [33] and Nazari *et al.* [34] reported that ETs in which the fundus was touched or embryos were <5 mm from the fundus resulted in an increased rate of ectopic gestation. The best site for embryo deposition in the uterus is 1–2 cm from fundus, and a reduced volume of medium including the embryo is also important for avoiding ectopic pregnancy.

Aspiration of cervical mucus, retained and expelled embryos

Poindexter *et al.* found that 4 of 46 patients (8.7%) displayed embryos in the cervix or on the speculum after reportedly routine transfers [35]. Use of soft catheters and complete aspiration of cervical mucus significantly reduced expulsion of the methylene blue dye [36].

Ultrasonographic examination before treatment

Ultrasonography provides precise information regarding length of the uterine cavity, length of the cervical canal and the presence of cervical angulations in relation to the uterine cavity. Ultrasonography is also very important for diagnosing fibroids that may be encroaching on the uterine cavity or distorting the cervical canal.

Avoiding the use of tenaculum

Lesny *et al.* reported that application of a tenaculum to the cervix during mock ET increased the occurrence of uterine contractions [37].

Leaving catheter in place

After pushing out embryos into the uterine cavity, pressure should be maintained on the syringe plunger until withdrawal of the catheter. One important precaution is the slow withdrawal of the catheter after injecting embryos. Rapid withdrawal of the catheter may create an area of negative pressure and result in the withdrawal of the embryo along with the catheter.

Use of antiprostaglandins

Release of prostaglandins may cause uterine contractions, potentially resulting in the expulsion of embryos into the cervical canal or Fallopian tubes. Use of antiprostaglandins is thus recommended.

Blastocyst transfer

Uterine junctional zone contractions progressively decrease with progression into the luteal phase, and this may be a contributing factor in the success of day 5 blastocyst-stage ET [31].

Other techniques

Pulling the transfer catheter 5 mm rapidly toward the operator while pushing the syringe slowly during ET appears to facilitate successful implantation, based on the experiences of the author.

Conclusion

This review has attempted to summarize various reports [7, 8, 38–42] supplemented with the perspectives of the author based on clinical experience.

Aspiration of oocytes

Oocyte collection technique - important points

1 Clean vagina of particulate matter before needle entry to reduce contamination of needle and vaginal bacterial count.
2 Focus vaginal ultrasound to maximize the size of each follicle so the needle can enter the center of the follicle.
3 Enter the follicle at the point of maximum diameter.
4 Commence aspiration before entering the follicle to prevent leakage.
5 Avoid excess aspiration pressure to prevent tearing the cumulus from the oocyte.
6 Flush follicles at low pressure.
7 Flush the aspirating system after emptying the first follicle to remove vaginal mucous and tissue.
8 Determine that the follicle is empty based on: a) several views on ultrasonography; and b) observation of aspirate in the tube.
9 Aspiration is easier if the ovary is fixed by firm manual pressure with 1 hand to reduce rotation of the ovary.
10 Operator should observe both ultrasound image of the follicle and tubal aspirate to coordinate movement of the ultrasound probe.
11 Color Doppler ultrasonography is recommended to decrease blood loss during oocyte aspiration.

Oocyte collection—difficulties

1 Transuterine needle puncture—minimize distance by manipulation of uterus or pressure on ovary—needle may bend or break.
2 Endometriosis fluid may be embryotoxic—leave endometriomas alone or aspirate endometrioma and flush cyst and needle repeatedly to clean.
3 Bleeding—Ovarian vessels: remove needle to stop bleeding; Iliac vein: remove needle gently; if rapid bleeding occurs, perform laparotomy; Vaginal bleeding: apply pressure for 2 min, and suture if bleeding continues.
4 Infection—use intravenous antibiotics for vaginal or cervical infection, or past history of pelvic infection or bowel or pelvic adhesions.
5 Hydrosalpinx—often contains embryotoxins, and removal before oocyte collection is preferable.
6 If hydrosalpinx is found at the time of oocyte collection, aspiration of all oocytes and subsequent aspiration of the hydrosalpinx is suggested, with repeated flushing using hypertoxic saline to reduce embryotoxins and further production of toxins.

Embryo transfer

1 The embryo catheter is passed through the internal cervical os and the embryo is delivered gently into the uterine cavity.

2 To avoid initiating uterine contractility with soft catheters, use gentle manipulation and avoid touching the fundus.
3 Proper evaluation of the uterine cavity and utero-cervical angulation should be performed using dummy ET and ultrasonography of the utero-cervical angulation and uterine cavity length.
4 Remove cervical mucus so that remnant mucus sticking to the catheter does not result in inadvertent removal of the embryo during catheter withdrawal.
5 The best site for embryo deposition in the uterus is 1–2 cm from the fundus, and a reduced volume of medium including embryos is also important for avoiding ectopic pregnancy.
6 Pulling the transfer catheter 5 mm rapidly toward the operator while pushing the syringe slowly during ET appears to facilitate successful implantation.

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