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To Flush Follicles during Egg Collection or Not

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Introduction

There is no doubt that the egg collection technique is an important part of *in vitro* fertilization (IVF). The question of whether to flush follicles during egg collection or not is still a controversial topic that has been hotly discussed since the introduction of IVF 40 years ago. In this chapter, an attempt is made to provide an answer to this question, providing evidence that supports flushing follicles in the proper way. Millions of oocytes could have been harvested, and thousands of babies could have been born, if the proper egg collection techniques had been used and a few simple rules of physics had been followed.

History of Egg Collection in *In Vitro* Fertilization

The first approach to ovum pickup (OPU) for IVF was taken using transabdominal laparoscopy (1). This approach was followed by the use of transabdominal, ultrasound-guided, oocyte retrieval (2). Suzan Lenz was the first physician to perform ultrasound-guided OPU in IVF in December 1980. She chose to use the transvesical follicle aspiration technique and conducted it at Rigs Hospital, Copenhagen, Denmark. Information about transvaginal oocyte retrieval with transvaginal aspiration under transabdominal ultrasound guidance was published in 1984 (3). Transvaginal OPU under transvaginal ultrasound guidance was introduced and reported in 1985 by Wikland and Hamberger (4) and has remained a standard of care for OPU since that time.

Brinsden remarked in 1992: "We believe that the use of vaginal ultrasound has enabled the technique of oocyte recovery to be refined down to the least invasive, least painful, most accurate, and most simple method that we are likely to be able to achieve in the foreseeable future" (5).

Since that time, the technique of using manual aspiration with syringes has been supplanted by that of electronic aspiration with pumps, which can maintain a steady aspiration pressure of about 120 mm Hg. Remarkably, the Rigs Hospital in Copenhagen is one of the few IVF centers at which a syringe instead of an aspiration pump is still used in OPU. Double-lumen needles or single-lumen needles with three-way valves have been widely used to optimize oocyte retrieval rates while flushing follicles.

A series of studies conducted over the past 30 years have compared the efficiency of single-lumen needles to double-lumen needles with the same outer diameter in terms of flushing follicles. The authors, however, did not consider the effects of the dramatically narrowed inner diameter of the inner needle on aspiration and the surrounding space required for flushing the double-lumen needles (6–11). Knight et al. (9) examined the effects of flushing and not flushing follicles on the total number of oocytes collected and pregnancy rates. Over 1139 treatment cycles, the authors found no statistical difference in the total number of oocytes collected or on pregnancy rates, but instead noted that increased operation time and anesthesia were required for the flushing group. On the basis of these observations, Knight concluded that flushing was "superfluous." For this reason, 80% of IVF doctors today use a single-lumen needle, and most do not flush follicles during egg collection.

State of the Art in Egg Collection

While 80% of researchers at IVF centers today "do not believe" in flushing follicles, 20% "do believe in the efficacy of flushing." As stated earlier, however, these beliefs—a mode of expression that is more accurately used in a religious or philosophic context—are based on a faulty conclusion that neglects the basic considerations of fluid dynamics that are taking place inside these needles. It is important to mention that a famous IVF clinic in Belgium, which has a noted reputation worldwide, routinely uses a 17-gauge single-lumen needle to flush follicles manually, injecting a flushing fluid into the silicone stopper of the needle system. This is a basis for concern, because the cumulus oocyte complex, which is already situated close to silicone stopper during this process, may be flushed back into that collapsed follicle if this technique is used (12).

Important Aspects of Fluid Dynamics Regarding Aspiration and Flushing Fluids in Pipes

In 1989, Reeves made an important statement regarding the behavior of fluids inside needles used in IVF during egg collection:

> The laws of physics applicable to a device such as an ovum pick up needle are those relating to the flow of fluid through pipes, in particular Poiseuillès law. This law takes in consideration the lumen diameter and the length of the conduit, in this case the length of the needle and the tube carrying the egg to the collecting container. The other parameters are the viscosity of the fluid and the force applied, in this case vacuum. Obviously, the success of the exercise is judged by the number of eggs, collected in relation to the number of follicles available; however, the law of physics used to collect the eggs are frequently ignored or not considered. (13)

The Poiseuillè law refers to the fact that at a constant driving pressure, the flow rate of a liquid through a capillary tube is directly proportional to the fourth power of the radius of the tube and inversely proportional to the length of the tube and viscosity of the liquid (Figure 10.1). For this reason, it is important for IVF doctors to carefully consider the implications of this law while flushing follicles for egg collection using either single- or double-lumen needles.

Another important consideration that must be taken is the *Reynolds number.* Described in the branch of physics concerned with the mechanics of fluids, the Reynolds number is a dimensionless number that gives a measure of the ratio of the inertial forces to the viscous forces and, consequently, quantifies the relative importance of these two types of forces for given flow conditions.

With regard to follicle flushing and egg collection, IVF physicians must consider both the Poiseuillè law and the Reynolds number, because a proper flushing effect can only be achieved if the effects of the flow rate and viscosity of the liquid being flushed *in relation to* the radius and the length of the needle

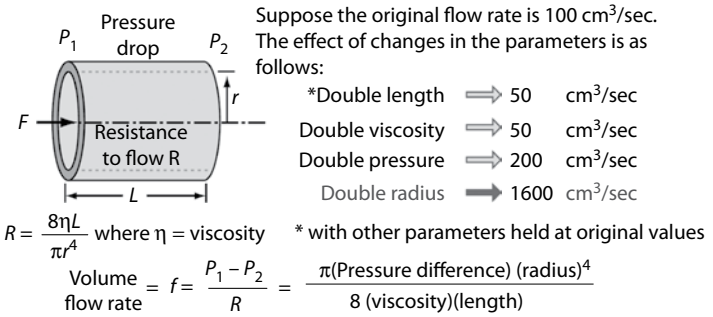

FIGURE 10.1 The Poiseuillè law. (From Hyperphysics, http://hyperphysics.phy-astr.gsu.edu, with permission.)

are considered *and* the effects of the inertial forces on the viscous forces in the particular flushing environment (i.e., the needle and the follicle being flushed). This flushing environment is defined by the needle used (ideally with an optimal inner diameter), the characteristics of the liquid being flushed, and the pulsating movement of the flushing syringe, which is activated either by hand or with the assistance of a mechanical or electronic flushing pump activated by the physician's foot. Because flushing has been summarily dismissed as "superfluous" by many physicians, this area of research has been neglected, and important possibilities for the optimization of egg collection in IVF have been overlooked (14).

Evidence in Favor of an Optimized Egg Collection System

By carefully considering the factors described earlier, an optimized egg collection system has been developed for use in my practice, which is based on considerations of physics. Specifically, this system includes the following components:

- A unique, single-lumen needle (STEINER-TAN needle) that enables follicles to be flushed through an opening as narrow as possible in order to minimize the patient's pain and the incidence of bleeding, but also achieve an optimal flushing effect. Details are shown below.
- A three-way stopcock is located close to the silicone stopper, which can be operated to change the direction of the fluid flow by changing its position from "aspiration" to "flushing." This stopcock is handled by an assistant manually or operated at a flushing pump, enabling the physician to change its position by activating a foot pedal without the need of assistance.
- The dead space present in the system has been minimized by 7 cm or the maximum length of the needle.
- A STEINER mechanical flushing pump (Figure 10.2) that can be activated by the physician's foot or a STEINER electronic aspiration and flushing pump that is combined in a single device (Figure 10.3) can be used. These pumps include an integrated warming element that ensures the constant temperature of the flushing fluid and prevents contamination and loss of temperature, which can occur if the flushing syringe is handheld.

Additional considerations that are involved with the use of the system include convincing colleagues that acoustic communication between the laboratory and operating room must be ensured. The biologist should have the ability to communicate commands in the case of poor responders, i.e., to continue flushing as long as granulosa cells are still found in the specimen.

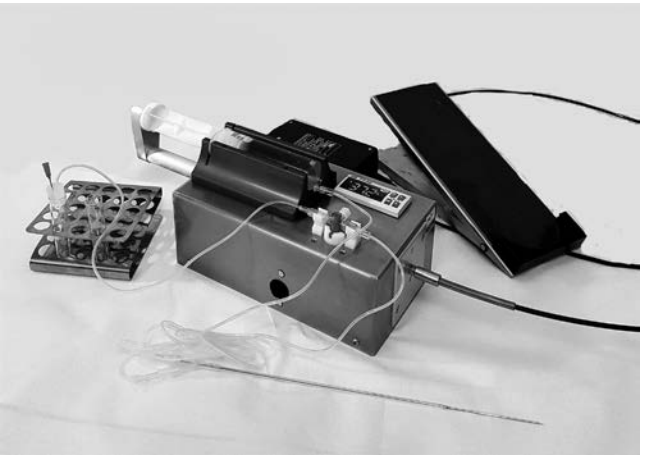

FIGURE 10.2 STEINER Mechanical Flushing Pump with Warming Element, shown here with STEINER-TAN needle, 17-gauge, with three-way valve clicked at pump.

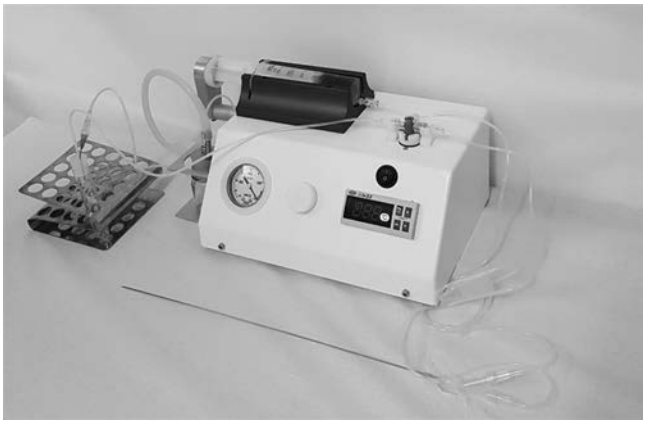

FIGURE 10.3 STEINER Combi Pump, shown here with STEINER-TAN needle 17-gauge classic.

There is sometimes confusion regarding the exact meaning of the term *flushing*. Flushing means completely refilling the follicle over a period of a few seconds. Flushing with a predefined volume, such as 3 cc as is performed in some centers, makes no logical sense because each flushing environment is inherently different. All follicles, even if there are 20 or more, should be flushed at least once to increase the chances of success (i.e., obtaining viable oocytes), which is currently not the standard in most IVF centers. The cumulative pregnancy rate resulting from the use of fresh or frozen oocytes should be the measure of IVF success.

STEINER-TAN Needle, a New Needle Technology

A single-lumen needle is inserted in plastic tubing starting at a position located 7 cm from the needle tip. After the follicle has collapsed, the flushing fluid is allowed to flow along the outside of the needle, passes through two drilled holes, and enters the lumen of the needle to refill the collapsed follicle (Figure 10.4). This needle optimizes the fluidic turbulence during both the aspirating and flushing processes (15) (Figure 10.5). In addition, the amount of dead space in the needle has been minimized to allow scientists to investigate the fluid of each follicle separately (16). The needle is optimally sharp (back bevel angle) and has an echo tip (Figure 10.6).

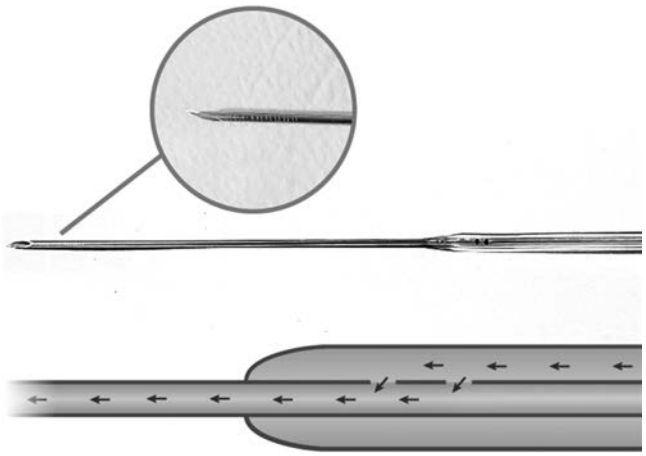

FIGURE 10.4 STEINER-TAN needle. For further information, please see the animation available on YouTube using the key words: Steiner computer animation.

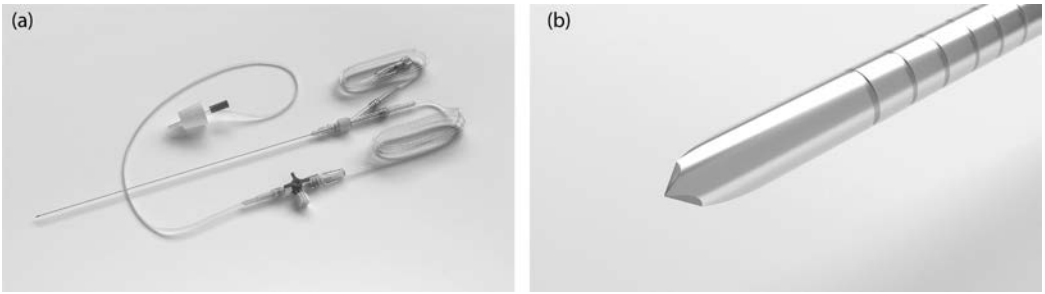

FIGURE 10.5 (a) STEINER-TAN needle: 17-, 19-, and 21-gauge quasi-DL, all with three-way stopcocks located close to the silicone stopper, to be used with a *special* needle guide (b). *Note*: Optimal back bevel angle.

FIGURE 10.6 STEINER-TAN Needle CLASSIC. Dead space length of needle (can be used with *any* needle guide).

STEINER Mechanical Flushing Pump

By activating the pedal, the physician can choose the velocity and amount of the flushing fluid by pressing a pedal with his or her foot. The warming element ensures that the flushing fluid is held at a constant temperature. This pump can be used with any DL needle available on the market or with STEINER-TAN needles.

STEINER Combi Pump

This is an electronic aspiration pump and flushing pump combined in a single device. The right pedal is pressed for aspiration; the left pedal is pressed for flushing. The warming element ensures that the flushing fluid is held at a constant temperature. The STEINER Combi Pump can be used with any DL needle available on the market or with STEINER-TAN needles. A helpful video is available on YouTube with the title "STEINER Combi Pump Egg Collection in IVF" (dated: December 7, 2019).

Comparison of the Flushing Effects in 17- and 19-Gauge STEINER-TAN Needles

We compared the flushing effects observed when using 17- and 19-gauge STEINER-TAN SL needles as to the flushing effects of 16-gauge VITROLIFE and 17-gauge COOK DL Needles (Figure 10.7). In order to obtain comparable results, we used the flushing line for aspiration. The comparison was carried out under conditions of constant vacuum (120 mm Hg), and an aspiration time of 20 seconds was used.

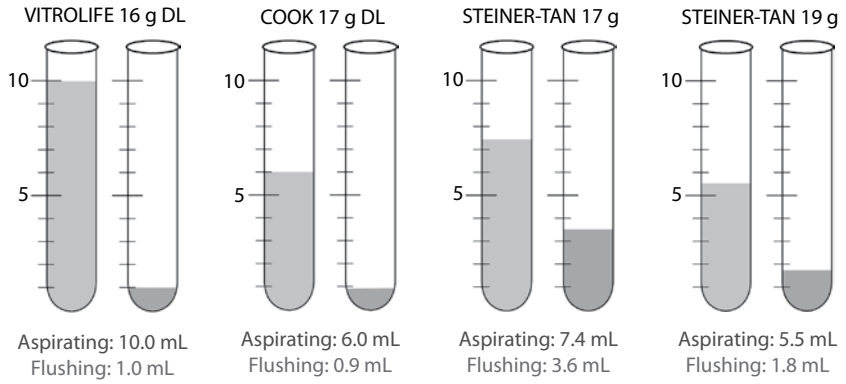

FIGURE 10.7 Comparison of the flushing effect in 17- and 19-gauge STEINER-TAN needles.

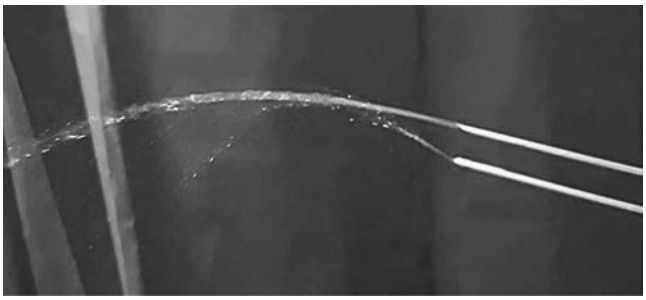

FIGURE 10.8 Comparison of flow characteristics between two needles: Upper needle: 17-gauge STEINER-TAN; lower needle: COOK 17-gauge DL. No linear flow was observed for the COOK 17-gauge DL due to technical characteristics of the needle. (For further information, please see the videos available on YouTube using the key words: Steiner, flushing.)

The 17-gauge STEINER-TAN SL needle demonstrated a nearly fourfold higher flushing capacity as compared to the 17-gauge COOK or 16-gauge VITROLIFE DL needles. The flushing capacity of the 19-gauge STEINER-TAN SL needle was twice that of the COOK DL 17-gauge and VITROLIFE DL 16-gauge needles. The flow characteristics for these needles were also compared and found to be extremely different (Figure 10.8).

How to Categorize the Flushing Effects of Different Needles

Before choosing to adopt a new needle technology, a flushing check can be easily performed so that an optimal decision can be made.

Empirical Flushing Check for Any SL or DL Needle

A horizontal flow of at least 15–20 cm is created using a 50 cc syringe filled with water (upper needle), ensuring that the collapsed follicle (diameter 18–20 mm) will be refilled (flushed) within approximately 5 seconds.

Pilot Study of Flushing versus Not Flushing Follicles

We conducted the first comparative study worldwide on the effects of flushing or not flushing follicles, with the primary goal to examine the flushing effects using a single needle with a single follicle. Studies that had been conducted previously had always compared the flushing effects of different needles and not

TABLE 10.1

Pilot Study—Flushing versus Not Flushing Follicles

Characteristics	Number	Percent (%)	Cumulative Percentage
No oocytes aspirated	204/543	(37.6)	
Oocytes aspirated	339/536	(62.4)	
Without flushing	152/339	(44.8)	(44.6)
Flushing 2×	158/339	(46.6)	(91.4)
Flushing 4×	29/339	(8.6)	(100.0)

nonflushing versus flushing per se. We used an 18-gauge STEINER-TAN needle with an outer diameter of 1.2 mm and an inner diameter of 0.8 mm, as well as 90 cm of tubing with an inner diameter of 1.5 mm. Because the needle had a dead space of needle of only 7 cm, it was possible to compare effects of flushing versus not flushing the follicle with a single needle for the first time. After the follicle had collapsed, it was possible to flush the proximal three-quarters of the length of the needle and tubing using a gentle flushing method without refilling follicle using this STEINER-TAN needle. At the same time, the aspiration was turned on. Each follicle was aspirated and flushed quasi-selectively (16). We punctured 543 follicles in 31 patients (averaging 17.5 follicles per patient, DM of follicles were 10 mm and larger), and 339 oocytes were aspirated.

Table 10.1 shows the results of the study: the number of oocytes retrieved could be more than doubled by flushing the follicle two times.

Case Report—Ultrasoft Stimulation IVF: Ovarian Hyperstimulation Syndrome Free IVF in Future PCO Patients?

Patients with polycystic ovary syndrome (PCO) are one of most important challenges in assisted reproductive technology (ART) due to the high incidence of severe side effects like ovarian hyperstimulation syndrome (OHSS). This case report demonstrates how we can perhaps avoid this sometimes life-threatening complication for our patients.

The patient was aged 29 years, with severe PCO, anti-Müllerian hormone (AMH) 7.5 ng/mL right ovary approximately 50, left ovary at least 30 antral follicles.

Treatment was as follows: (**Previous cycle**: Oral contraceptive)

Cycle day 3: 150 Mikrogramm Elonva (Corifollitropin alfa)

Day 9: 1. Egg Collection: Canceling egg collection after aspiration and flushing half of follicles at right ovary due to sporadic granulosa cells only and 3 retrieved immature oocytes (1 M I, 2× GV)

Evening of day 9: Agonist triggering ovulation with 2 Amp. DECAPEPTYL 0.1 mg/1 mL (100 Mikrogramm Triptorelinacetat)

Day 11: 2. Egg Collection: 36 hours after agonist trigger. Out of both ovaries 16 oocytes, 13 MII for intracytoplasmic sperm injection (ICSI).

(Result of 1. Egg collection: 1× *in vitro* maturation (IVM) ICSI after 24 hours IVM: 1× 3PN Result of 2. Egg Collection: out of 13× ICSI on day of Egg Collection: 10× 2PN)

Day 5: 5 Blastocysts

Day 6: 2 more Blastocysts

Freeze-all policy due to unsynchronized endometrium.

Egg Collection Technique as follows: Under mild sedation, STEINER-TAN needle 19-gauge for flushing two times each follicle, STEINER Scraper (110° automatically rotating "scraping" movement of needle during aspiration). Please watch YouTube video STEINER Scraper.

Hypothesis: Using Corifollitropin 150 as the only medication for ultrashort stimulation in PCO patients in combination with early "triggering ovulation" with agonist using flare-up effect of pituitary gland

and freeze-all policy is a promising solution in order to avoid any degree of OHSS. Alternatively, in countries where Elonva is not yet approved by the food and drug regulatory agency: recombinant follicle-stimulating hormone 150 from day 3 until day 8.

This case report of ultrasoft stimulation IVF with 7 blastocysts out of 10 fertilized oocytes should be the trigger to replace conventional IVF and IVM in PCO patients in the future and should inspire further studies.

Conclusion

Through extensive practical experience and scientific research, we can conclude that physicians working in the field of IVF should focus on developing new reproductive technologies and products to improve the pregnancy rates of the clients.

Vaughn et al. (17) stated that IVF physicians can increase cumulative pregnancy rates (from fresh and frozen cycles) in IVF by 8% with every additional egg harvested. This finding is noteworthy and should encourage all physicians working in this field to invest efforts in optimizing the percentage of harvested eggs/follicles by exploring such technological advances. Physicians should critically rethink the standard egg collection technique used in their clinics and use optimized egg collection systems, rather than investing more money in technologies that have not been shown to improve the clinical pregnancy rate. In the words of Albert Einstein, "We cannot solve our problems with the same level of thinking that created them."

Disclosure

The author of this chapter founded the company IVFETFLEX.COM with the brand name *IVF-future* to develop and market the optimized egg collection technology mentioned in this chapter.

Addendum

Protocol for Economic Flushing Medium

Note: Pre-warm overnight in an incubator.

Version (a)

500 mL Minimum Essential Medium (MEM) with Earle's Balanced Salts with L-glutamine
VWR (0043 1 97002444) Art. L0415–500
0.5 mL penicillin G-natrium, 1 MEGA IE
(amp. with 5 mL dissolved in aqua bidest; use 0.5 mL and the remainder can be frozen in portions)
0.125 mL heparin immuno 5000 I.E./mL (pharmacy)
11 mL aqua bidest
L-glutamine, penicillin, heparin, and sterile aqua bidest are combined, filtered, and mixed with MEM.

Version (b)

500 mL NaCl + 0.125 mL heparin

REFERENCES

1. Steptoe PC, Edwards RG. Laparoscopic recovery of preovulatory human oocytes after priming of ovaries with gonadotrophins. *Lancet*. 1970;1(7649):683–9.
2. Lenz S, Lauritsen JG, Kjellow M. Collection of human oocytes for *in vitro* fertilisation by ultrasonically guided follicular puncture. *Lancet*. 1981;1(8230):1163–4.

3. Gleicher N, Friberg J, Fullan N, Giglia RV, Mayden K, Kesky T, Siegel I. Egg retrieval for *in vitro* fertilisation by sonographically controlled vaginal culdocentesis. *Lancet*. 1983;2(8348):508–9.
4. Wikland M, Enk L, Hamberger L. Transvesical and transvaginal approaches for the aspiration of follicles by use of ultrasound. *Ann N Y Acad Sci*. 1985;442:182–94.
5. Brinsden PR, Rainsbury PA. (editors.) *In Vitro Fertilization and Assisted Reproduction*. New York, NY: Parthenon Publishing Group; 1992.
6. Scott RT, Hofmann GE, Muasher SJ, Acosta AA, Kreiner DK, Rosenwaks Z. A prospective randomized comparison of single- and double-lumen needles for transvaginal follicular aspiration. *J In Vitro Fert Embryo Transf.* 1989;6(2):98–100.
7. Kingsland CR, Taylor CT, Aziz N, Bickerton N. Is follicular flushing necessary for oocyte retrieval? A randomized trial. *Hum Reprod*. 1991;6(3):382–3.
8. Waterstone JJ, Parsons JH. A prospective study to investigate the value of flushing follicles during transvaginal ultrasound-directed follicle aspiration. *Fertil Steril*. 1992;57(1):221–3.
9. Knight DC, Tyler JP, Driscoll GL. Follicular flushing at oocyte retrieval: A reappraisal. *Aust N Z J Obstet Gynaecol*. 2001;41(2):210–3.
10. Levens ED, Whitcomb BW, Payson MD, Larsen FW. Ovarian follicular flushing among low-responding patients undergoing assisted reproductive technology. *Fertil Steril*. 2009;91(4 Suppl):1381–4.
11. Bagtharia S, Haloob AR. Is there a benefit from routine follicular flushing for oocyte retrieval? *J Obstet Gynaecol*. 2005;25(4):374–6.
12. Méndez Lozano DH, Fanchin R, Chevalier N, Feyereisen E, Hesters L, Frydman N, Frydman R. [The follicular flushing duplicate the pregnancy rate on semi natural cycle IVF] [Article in French]. *J Gynecol Obstet Biol Reprod (Paris)*. 2007;36(1):36–41.
13. Reeves G. Letters to the editor. *J In Vitro Fert Embryo Transf.* 1989;6(6):353–4.
14. Sunkara SK, Rittenberg V, Raine-Fenning N, Bhattacharya S, Zamora J, Coomarasamy A. Association between the number of eggs and live birth in IVF treatment: An analysis of 400 135 treatment cycles. *Hum Reprod*. 2011;26(7):1768–74.
15. Steiner HP. Optimizing technique of follicular aspiration and flushing. In: Chávez-Badiola A, Allahbadia GN, editors. *Minimal Stimulation IVF—Milder, Mildest or Back to Nature*. New Delhi: Jaypee Brothers Medical Publishers; 2011: 98–102.
16. Schenk M, Huppertz B, Obermayer-Pietsch B, Kastelic D, Hörmann-Kröpfl M, Weiss G. Biobanking of different body fluids within the frame of IVF—A standard operating procedure to improve reproductive biology research. *J Assist Reprod Genet*. 2017;34(2):283–90.
17. Vaughan DA, Leung A, Resetkova N, Ruthazer R, Penzias AS, Sakkas D, Alper MM. How many oocytes are optimal to achieve multiple live births with one stimulation cycle? The one-and-done approach. *Fertil Steril*. 2017;107(2):397–404.