CEOG Clinical and Experimental Obstetrics & Gynecology

The influence of different semen volumes on pregnancy rates in unexplained infertility

P. Costur Filiz[1], E. Doger[2], Y. Cakiroglu[2], A. Saribacak[3], E. Caliskan[4], S. Filiz[2]

[1] Department of Obstetrics and Gynecology, VM Medical Park Kocaeli Hospital, Kocaeli; [2] Faculty of Medicine, Kocaeli University, IVF Center, Kocaeli; [3] Department of Urology, VM Medical Park Kocaeli Hospital, Kocaeli

[4] Department of Obstetrics and Gynecology, Bahçeşehir University, Faculty of Medicine, Istanbul (Turkey)

Summary

Purpose: The purpose of this study was to determine the efficacy of low- and large-volume techniques of intrauterine insemination (IUI) on pregnancy rates in unexplained infertility couples. *Materials and Methods:* This retrospective study was performed in a single center over a 33-month period which included a total of 272 couples that underwent 272 IUI cycles that were divided into two groups. Low-volume group underwent 0.5 ml of inseminated semen; large-volume group underwent four ml of inseminated semen. The clinical pregnancy rates per cycle in each age group were evaluated. *Results:* In the large-volume group, clinical pregnancy rates were higher. In ≤ 34 age groups, clinical pregnancy rates were higher in large-volume group while in > 35 age group, clinical pregnancy rates were higher in low-volume group. *Conclusion:* This study clearly demonstrated the effectiveness of large-volume technique on IUI success in < 35 age women with unexplained infertility.

Key words: Intrauterine insemination; Low inseminated volume; Large inseminated volume; Clinical pregnancy.

Introduction

Intrauterine insemination (IUI) is the first step in assisted reproductive technique in many infertility centers for mild male factor, which is simple, non-invasive, and less expensive compared to in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) techniques [1]. Some indications for performing IUI are unexplained infertility, mild male factor, genital tract anomalies, erectile dysfunction, some cervical factors, and moderate endometriosis [2].

IUI success rate depends on several factors, such as sperm quality, post-wash total progressive motile sperm count (TPMSC), female age, number of cycles, cause and duration of infertility, drugs, insemination time, and IUI techniques [3, 4]. Although semen analysis, thereby determining the semen parameters, is the first critical point for IUI success; they are all controversial and there is no concensus on these parameters and also on sperm preparation and transfer techniques [5-8]. For IUI success, TPMSC varies from 0.3×10^6 to 20×10^6 [7]. So far some sperm preparation methods, such as swim-up and migration, density/discontinuous gradient centrifugation (DGC), glass wool filters, and recently, microfluidic sperm sorting, have been used. Many types of transfer catheters and transfer techniques were used such as transferring sperm to uterus with low volume which are known as traditional IUI or with large volume which are known as fallopian perfusion [9, 10]. Currently, conflicting results have also been observed. Although, Kahn *et al.* described a new method for IUI while using large semen volume (four ml) described as fallopian sperm perfusion (FSP) and obtained better pregnancy rates compared with low volume (0.5 ml) [11, 12], while other studies did not detect any advantages [13, 14]. Trout *et al.* obtained high pregnancy rates with large-volume only in unexplained cases [15]. Ok *et al.* did not find any significant relationship between post-wash inseminated semen volume (0.3, 0.4, or 0.5 ml) and the pregnancy rates [16]. In the literature, many studies describe various parameters and techniques for IUI success, but only a few number of study performed on volume.

The aim of this retrospective study was to compare the efficacy of low- and large-volume techniques of IUI on pregnancy rates in unexplained infertility couples.

Materials and Methods

This retrospective single center randomized controlled study comparing the effectiveness of low- and large-volume IUI transfer techniques on pregnancy rates in couples undergoing IUI cycles with unexplained infertility or moderate male subfertility.

A total of 272 couples underwent 272 IUI cycles from January 2010 to September 2012 in the Kocaeli University Faculty of Medicine IVF Center were included in this study. Inclusion criteria for women were up to 39-years-old (a range from 23 to 39 years) with normal bilateral fallopian tubes confirmed by hysterosalpingogram (HSG), unexplained infertility with no specific etiology, and for men with normal semen analysis according to WHO criteria [17]. Couples with tubal factors, severe endometriosis, endocrine disorders, more than three follicles on hCG

Revised manuscript accepted for publication April 11, 2016

day, and severe male factor were excluded.

The couples were divided into two groups as low-volume group (n=116) and large-volume group (n=156), according to the IUI technique. Women's age in low-volume group were ≤ 29 years (n=36), 30-34 years (n=30), and 35-39 years (n=50), as well as women's age in large-volume group were ≤ 29 years (n=48), 30-34 years (n=80), and 35-39 years (n=28). The study protocol was approved by the Faculty Ethics Committee.

All patients underwent baseline transvaginal ultrasonography (TVU) on day 2 or 3 of the menstrual cycle and ovarian stimulation started with a dose of 75-100 IU recombinant FSH. Serum hormone levels were measured and the follicular development and also the thickness of endometrium was evaluated by TVU on seventh to eighth day of cycles, and if necessary, the doses were organized according to the ovarian response. This evaluation was repeated after two or three days depending on follicular development. Cycles were triggered with a single dose of 250 μcg recombinant hCG when at least one dominant follicle had reached 18 mm in diameter. IUI was scheduled 36 hours after recombinant hCG administration.

Semen samples were obtained by masturbation in sterile plastic containers after three to five days of sexual abstinence on the IUI transfer day. The manipulation of semen samples were carried out inside the laminar flow. Seminal parameters (basal sperm morphology, count, and motility) were analyzed by two researchers after the semen liquefaction. First, 10-μl sample was placed on a prewarmed (37°C) Makler counting chamber to evaluate the parameters by using phase contrast microscope. After seminal analysis, semen samples were processed using a density gradient and sperm wash medium at room temperature. Using a sterile pipette, one ml of 90% gradient solution was added to a 15-ml Falcon tube to form a lower layer and followed by one ml of 50% gradient solution added for upper layer. After the tube was incubated at 37°C for 15 minutes, two ml of liquefied semen was gently added to the tube and then centrifuged at 1,600 rpm for ten minutes. After centrifugation, the supernatant was carefully removed without disturbing the pellet. Three ml sperm washing solution was added onto the pellet, resuspended, and centrifuged for ten minutes at 1,600 rpm. The supernatant was then removed and the final pellet was placed into a new Falcon tube with a pipette and 0.2−0.3 ml (a total of 0.5 ml) or 3.7−3.8 ml (a total of four ml) sperm washing medium was added on the pallette. The tube was then incubated in the incubator at 37°C and 5% CO_2 for insemination.

IUI was performed using an intrauterine catheter after 36 hours of hCG administration. The patients rested for 20 minutes in a supine position after insemination. For luteal phase progesterone support, 600 mg/day vaginal progesterone was initiated on the same day after insemination until to pregnancy test. Serum β-hCG levels were determined two weeks after post insemination for diagnosis of pregnancy. If the serum β-hCG level was > 40 IU/L, it was considered as biochemical pregnancy. Clinical pregnancy was confirmed by TVU defined by the presence of gestational sac after one week of positive β-hCG and then embryonic heartbeat was assessed three weeks later. If pregnancy occurred, patients continued to receive progesterone support up to eighth week of gestation.

In this study, the primary outcome was clinical pregnancy. Statistical analysis of the data was performed using SPSS software (version 16). Clinical characteristics, basal semen parameters, and pregnancy rates were expressed as mean ± SD and the comparison of the groups were performed by Mann-Whitney U-test. A $p < 0.05$ was considered to be statistically significant for all tests.

Table 1. — *Clinical characteristics of patients that underwent IUI.*

	Low-volume group (n=116)	Large-volume group (n=156)	*p*-value
Female age (years)	29.12±5.02	30.01±5.08	0.152
Male age (years)	32.12±6	33.23±5.4	0.109
Duration of infertility (years)	3.83±3	4.10±2.83	0.456
Unexplained infertility (%)	98 (84.5%)	128 (82.1%)	0.597
Male factor (%)	18 (15.5%)	28 (17.9%)	0.597
Days of stimulation	10.37±2.24	10.3±2.19	0.817
Number of follicles (≥ 17mm) on the hCG day	1.43±0.64	1.45±0.63	0.760
Endometrial thickness on the hCG day (mm)	10.03±2.34	9.96±2.31	0.787

Results

Two hundred and seventy-two controlled ovarian stimulation cycles for IUI were performed in 272 patients. The clinical characteristics of patients that underwent IUI are shown in Table 1. The median age of women in low- and large-volume groups was 29.12 ± 5.02 and 30.01 ± 5.08, years, respectively.

In low-volume group, 98 patients (84.5%) had unexplained infertility and 18 patients (15.5%) had moderate male factor. In large-volume group, 128 patients (82.1%) had unexplained infertility and 28 patients (17.9%) had moderate male factor. As shown in Table 1, the comparison of ages, duration of infertility, stimulation days, number of follicles, and the endometrial thickness on the hCG day did not show any statistical significance.

In Table 2, seminal parameters are shown. The authors observed that there was no significant difference between low- and large-volume groups according to basal sperm concentration, basal sperm motility, sperm morphology, progressive motility, and TPMSC.

Table 3 shows the number of pregnancies, pregnancy rates according to age and total pregnancies in both groups. The pregnancy rates per cycle showed a total significant difference between the studied large- and low-volume groups (30.8% and 19%, respectively, $p < 0.028$). Pregnancy rates per cycle were higher in ≤ 29 age group in the large-volume group compared to the low-volume group which was not statistically significant (25% and 16.7%, respectively, $p < 0.357$). In 30-34 age groups, pregnancy rates per cycle were statistically significant in the large-volume group in comparison to the low-volume group (40% and 13.3%, respectively, $p < 0.008$). However, pregnancy rates per cycle were higher in 35-39 age group in the low-volume group (24%) in comparison to the large-volume group (14.3%) which was not statistically significant ($p < 0.308$).

Table 2. — *Comparison of seminal parameters of both groups.*

	Low-volume group (n=116)	Large-volume group (n=156)	*p*-value
Semen volume (ml)	2.56±0.9	2.42±0.87	0.178
Basal sperm concentration ($\times 10^6$/ml)	45.69±17.5	42.5±19.4	0.159
Basal sperm motility (%)	60.17±10.23	58.25±11.06	0.146
Progressive motility (%)	48.63±9.84	50.25±10.84	0.207
Morphology (%)	4.68±1.75	4.89±1.34	0.285
TPMSC ($\times 10^6$/ml)	28.5±13.07	26.04±12.12	0.111

TPMSC: total progressive motile sperm count.

Table 3. — *Comparison of pregnancy rates for low- and large-volume groups.*

	Low-volume group (n=116) Clinical pregnancy	Large-volume group (n=156) Rates per cycle (%)	*p*-value
≤ 29 age group	6/36 (16.7%)	12/48 (25%)	0.357
30-34 age group	4/30 (13.3%)	32/80 (40.0%)	0.008*
35-39 age group	12/50 (24%)	4/28 (14.3%)	0.308
Total pregnancy rate (%)	22/116 (19%)	48/156 (30.8%)	0.028*

*Statistically significant.

Discussion

IUI is a simple, inexpensive, and non-invasive first-step technique for infertile couples in comparison with other assisted reproductive techniques [18, 19]. It has been widely used in infertile couples in many countries where the infertility cause is male subfertility, unexplained infertility, and mild endometriosis [20]. Although National Institute for Health and Care Excellence (NICE) clinical guidelines [21] do not recommended IUI for unexplained infertility, mild endometriosis, and mild male factor, three or four inseminations before IVF treatments have been usually performed in most IVF centers and described as a patient-friendly strategy in comparison to IVF/ICSI [22, 23]. In fact, as already discussed, IUI success parameters are all controversial. In most study, success rate of the IUI differs and depends on several factors, such as various infertility factors, duration of infertility, number of previously performed cycles, woman's age, ovarian stimulation protocols, semen quality and some seminal parameters, semen preparation techniques, follicle number, endometrial thickness, etc. As discussed, IUI success parameters were investigated in many studies, however, only a few studies comparing the inseminated volumes have been found. Traditional or classical IUI implies the using of low semen volume as 0.5−1 ml [6, 16, 24] while the large semen volume (FSP) implies the use of three- to four-ml semen volume [11, 12, 14, 15].

In this study, the authors aimed to compare the efficacy of low- and large-volume techniques of IUI on pregnancy rates. The groups of the study included similar ages, duration of infertility, stimulation days, number of follicles, and the endometrial thickness on the hCG day which did not show any statistical significance. Majority of the patients had unexplained infertility and a few had moderate male factor subfertility in both groups, and also underwent similar ovarian stimulation. Basal sperm concentration, basal sperm motility, progressive motility, sperm morphology, and TPMSC did not show any statistical significance. Herein, the clinical pregnancy rates acquired by 272 ovarian stimulated IUI cycles are described.

In the present study, women's age were 29.12 ± 5.02 in low-volume group and 30.01 ± 5.08 in large-volume group which was not statistically different and underwent similar ovarian stimulation protocol. In ≤ 29 and 30-34 age groups, pregnancy rates were higher in large-volume group (25% and 40%) compared with low-volume group (16.7% and 13.3%), however, pregnancy rates were higher in low-volume group in 35-39 age group in comparison to large-volume group (24% and 14.3%, respectively). In a prospective randomized study with 60 patients diagnosed as unexplained infertility, Kahn *et al.* achieved 26.9% pregnancy rates per cycle with large-volume (four ml) compared with classical IUI (9.8%) [25]. Furthermore, they received higher than 25% pregnancy rates with large-volume in donor insemination programme [11]. Fanchin *et al.* studied 74 infertile women aged 20 to 38 years undergoing 100 cycles of COH and achieved 40% clinical pregnancy rates per cycle with large-volume (four ml) in comparison to 0.2 ml volume (20%) [26]. In 1996, Mamas studied unexplained infertility diagnosed in 104 couples undergoing 202 cycles and obtained higher clinical pregnancy rates with large volume (four ml) compared with 0.5 ml standard IUI (26.36% and 11.95%, respectively) [27]. In a prospective randomized study and meta-analysis of the literature, high pregnancy rates (40%) were detected only in patients with unexplained infertility [15]. Similar to these aforementioned studies, Ricci *et al.* compared the effectiveness of large-volume and standard IUI in 132 cycles with unexplained infertility and achieved high ongoing pregnancy rates with large volume (21.2% and 7.6%, respectively) [28]. Eventually, a systematic analysis based on a Cochrane review revealed that although the results in pregnancy rate per couple revealed no statistically significant difference between large-volume and classic IUI, subgroup analysis revealed that couples suffering from unexplained subfertility clearly benefit from large-volume over classic IUI [29]. Although the present data confirmed these reports in < 35 age groups, it is possible that the sample size may not be sufficient to detect better results for > 35 age group and may be associated with unexplained female factors. Nevertheless, Karande *et al.* and Do Amaral *et al.* when they compared the results of large-volume (four and three ml, respectively) and classic IUI, did not detect any advantage of large-volume [14, 30]. Also, Cantineau *et al.* in a meta-analysis [31] and in its updated version

[32] claimed that no clear evidence suggests any difference between classic IUI and large-volume with respect to their effectiveness and safety for treating couples with non-tubal subfertility and recommended additional research because of a high level of uncertainty is evident in the findings.

Demir *et al.* found that the pregnancy rate was higher when the woman were < 25-years-old, total number of motile sperm was $>10\times10^6$, and when sperm morphology was > 4% [33], but Araujo *et al.* found no significant difference in age in both pregnancy and non-pregnancy groups [34]. Although Cohlen *et al.* noticed that IUI offers couples with male subfertility benefit over timed intercourse, both in natural and controlled ovarian hyperstimulation (COH) cycles [35], it was updated as there was insufficient evidence of effectiveness to recommend or advise againist IUI, with or without ovarian hyperstimulation above timed intercourse [36]. van Rumste *et al.* demonstrated the association of ovarian stimulation with increased pregnancy rates in IUI and concluded that IUI with ovarian stimulation should not aim for more than two follicles because of increased multiple pregnancy [37].

Semen quality and seminal parameters were investigated in many studies and claimed its importance in attaining high pregnancy rates in couples undergoing IUI. Studies have demonstrated that sperm motility is considered a crucial prognostic factor for IUI success [38-40]. van Weert *et al.* indicated the importance of inseminating motile sperm count at cut-off levels between 0.8 and five million in a meta-analysis [41]. However, Luco *et al.* indicated that total motile sperm count was not to be a predictor of pregnancy [23]. Sperm morphology was also investigated in many studies [42, 43]. Ombelet *et al.* revealed that the literature did not give powerful evidence on the relationship between sperm quality and IUI success but nevertheless offered as inseminated motile sperm >one million, sperm morphology using strict criteria > 4%, total motile sperm count 5-10 million, and total motility > 30% in a systematic review [44]. The present data fulfilled the results of the Ombelet *et al.* review.

Sperm preparation techniques on IUI prognosis were also controversial. Up-to-date sperm preparation methods have been described while the most commonly used methods are DGC and swim-up. Th present authors performed DGC in all samples of both groups. Although some studies showed that sperm with better DNA and chromatin integrity were associated with higher fertilization potential revealed by DGC [45, 46], some studies demonstrated that clinical pregnancy rates were higher in motile spermatozoa obtained by swim-up method [47]. It was concluded that no significant difference was demonstrated comparing the efficiency of the swim-up and gradient methods [48] or none of the sperm preparation methods were found to be superior to any other [49].

Conclusion

In conclusion, although the literature in this field is controversial, this study clearly demonstrated the effectiveness of large-volume technique on IUI success in < 35-year-old women with unexplained infertility, which was especially significantly difference in the 30-34 age group.

References

[1] Hughes E.G.: "The effectiveness of ovulation induction and intrauterine insemination in the treatment of persistent infertility: a meta-analysis". *Hum. Reprod.,* 1997, *12*, 1865.
[2] Marchetti C., Dewailly D.: "Intrauterine insemination: indications and methods". *Rev. Prat.,* 2006, *56*, 500.
[3] Nikbakht R., Saharkhiz N.: "The Influence of sperm morphology, total motile sperm count of semen and the number of motile sperm inseminated in sperm samples on the success of intrauterine insemination". *Int. J. Fertil. Steril*., 2011, *5*, 168.
[4] Marviel P., Heraud M.H., Grenier N., Lourdel E., Sanguinet P., Copin H.: "Predictive factors for pregnancy after intrauterine insemination (IUI): an analysis of 1038 cycles and a review of the literature". *Fertil. Steril*., 2010, *93*, 79.
[5] Campana A., Sakkas D., Stalberg A., Bianchi P.G., Comte I., Pache T., Walker D.: "Intrauterine insemination: evaluation of the results according to the woman's age, sperm quality, total sperm count per insemination and life table analysis". *Hum. Reprod.,* 1996, *11*, 732.
[6] Zhao Y., Vlahos N., Wyncott D., Petrella C., Garcia J., Zacur H., Wallach E.E.: "Impact of semen characteristics on the success of intrauterine insemination". *J. Assist. Reprod. Genet*., 2004, *21*, 143.
[7] Van Weert J.M., Repping S., Von Voorhis B.J., Bossuyt P.M., Mol B.W.: "Performance of the post-wash total motile sperm count as a predictor of pregnancy at the time of intrauterine insemination: a meta-analysis". *Fertil. Steril*., 2004, *82*, 612.
[8] Dorjpurev U., Kuwahara A., Yano Y., Taniguchi T., Yamamoto Y., Suto A., *et al.*: "Effect of semen characteristics on pregnancy rate following intrauterine insemination". *J. Med. Invest*., 2011, *8*, 127.
[9] Franco Junior J.G., Baruffi R.L., Mauri A.L., Stone S.C.: "Radiologic evaluation of incremental intrauterine instillation of contrast material". *Fertil. Steril*., 1992, *58*, 1065.
[10] Kahn J.A., Sunde A., Von During V., Sordal T., Molne K.: "Intrauterine insemination". *Ann. NY. Acad. Sci*., 1991, *626*, 452.
[11] Kahn J.A.,Von During V., Sunde A., Sordal T., Molne K.: "Fallopian tube sperm perfusion used in a donor insemination programme". *Hum. Reprod*., 1992, *7*, 19.
[12] Kahn J.A., Von During V., Sunde A., Sordal T., Molne K.: "Treatment of unexplained infertility. Fallopian tube sperm perfusion (FSP)". *Acta. Obstet. Gynecol. Scand*., 1993, *72*, 193.
[13] Nuojua-Huttunen S., Tomas C., Bloigu R., Tuomivara L., Martikainen H.: "Intrauterine insemination treatment in subfertility: An analysis of factors affecting outcome". *Hum. Reprod*., 1999, *14*, 698.
[14] Do Amaral V.F., Ferriani R.A., Dos Reis R.M., De Sala M.M., De Moura M.D.: "Effect of inseminated volume on intrauterine insemination". *J. Assist. Reprod. Genet*., 2001, *18*, 413.
[15] Trout S.W., Kemmann E.: "Fallopian sperm perfusion versus intrauterine insemination: A randomized controlled trial and metaanalysis of the literature". *Fertil. Steril.*, 1999, *71*, 881.
[16] Ok E.K., Dogan O.E., Okyay R.E., Gulekli B.: "The effect of post-wash total progresivve motile sperm count and semen volume on pregnancy outcomes in intrauterine insemination cycles: a retrospective study". *J. Turkish-German Gynecol. Assoc*., 2013, *14*, 142.
[17] WHO Health Organization.: "WHO Laboratory Manual for the Examination and Processing of Human Semen", 5th ed. Cambridge: Cambridge University Press, 2010.
[18] Zhao Y., Vlahos N., Wyncott D., Petrella C., Garcia J., Zacur H., Wallach E.E.: "Impact of semen characteristics on the success of intrauterine insemination". *J. Assist. Reprod. Genet*., 2004, *21*, 143.

[19] Cohlen B.J.: "Should we continue performing intrauterine inseminations in the year 2004?" *Gynecol. Obstet. Invest*., 2005, *59*, 3.
[20] Goverde A.J, McDonnell J., Vermeiden J.P., Schats R., Rutten F.F., Schoemaker J.: "Intrauterine insemination or in-vitro fertilisation in idiopathic subfertility and subfertility: a randomised trial and cost-effectiveness analysis". *Lancet*, 2000, *355*, 13.
[21] NICE Guidelines.: "Fertility: assessment and treatment for people with fertility problems". Clinical guidelines, CG156-Issued: February 2013.
[22] Pennings G., Ombelet W.: "Coming soon to your clinic: patient-friendly ART". *Hum. Reprod*., 2007, *22*, 2075.
[23] Luco S.M., Agbo C., Behr B., Dahan M.H.: "The evaluation of pre and post processing semen analysis parameters at the time of intrauterine insemination in couples diagnosed with male factor infertility and pregnancy rates based on stimulation agent. A retrospective cohort study". *E. J. Obstet. Gynecol. Reprod. Biol*., 2014, *179*, 159.
[24] Karamahmutoglu H., Erdem A., Erdem M., Mutlu M.F., Bozkurt N., Oktem M, *et al.*: "The gradient technique improves success rates in intrauterine insemination cycles of unexplained subferile couples when compared to swim up technique; a prospective randomized study". *J. Assist. Reprod. Genet*., 2014, *31*, 1139.
[25] Kahn J.A., Sunde A., Koskemies A., von Düring V., Sordal T., Christensen F., Molne K.: "Fallopian tube sperm perfusion (FSP) versus intra-uterine insemination (IUI) in the treatment of unexplained infertility: a prospective randomized study". *Hum. Reprod*., 1993, *8*, 890.
[26] Fanchin R., Olivennes F., Righini C., Hazout A., Schwab B., Frydman R.: "A new system for fallopian tube sperm perfusion leads to pregnancy rates twice as high as standard uterine insemination". *Fertil. Steril*. 1995, *54*, 505.
[27] Mamas L.: "Higher pregnancy rates with a simple method for fallopian tube sperm perfusion, using the cervical clamp double nut bivalve speculum in the treatment of unexplained infertility: a prospective randomized study". *Hum. Reprod*., 1996, *11*, 2618.
[28] Ricci G., Nucera G., Pozzobon C., Boscolo R., Giolo E., Guaschino S.: "A simple method for fallopian tube sperm perfusion using a blocking device in the treatment of unexplained infertility". *Fertil. Steril*., 2001, *76*, 1242.
[29] Cantineau A.E., Heineman M.J., Al-Inany H., Cohlen B.J.: "Intrauterine insemination versus Fallopian tube sperm perfusion in non-tubal subfertility: a systematic review based on a Cochrane review". *Hum. Reprod*. 2004, *19*, 2721.
[30] Karande V.C., Rao R., Pratt D.E., Balin M., Levrant S., Morris R., *et al.*: "A randomised prospective comparison between intrauterine insemination and fallopian sperm perfusion for the treatment of infertility". *Fertil. Steril*., 1995, *64*, 638.
[31] Cantineau A.E., Cohlen B.J., Heineman M.J.: "Intrauterine insemination versus fallopian tube sperm perfusion for non-tubal infertility". *Cochrane Database Syst. Rev*., 2009, *2*, CD001502.
[32] Cantineau A.E., Cohlen B.J., Heineman M.J., Marjoribanks J., Farquhar C.: "Intrauterine insemination versus fallopian tube sperm perfusion for non-tubal infertility". *Cochrane Database Syst. Rev*., 2013, *10*, CD001502.
[33] Demir B., Dilbaz B., Cinar O., Karadag B., Tasci Y., Kocak M., *et al.*: "Factors affecting pregnancy outcome of intrauterine insamination cycles in couples with favourable female characteristics". *J. Obstet. Gynecol*., 2011, *31*, 420.
[34] De Araujo L.F., de Aroujo Filho E., Facio C.L., Bossoni M.C., Machado-Paula L.A., Corrente J.E., et al.: "Efficacy of sperm motility after processing and incubation to predict pregnancy after intrauterine insemination in normospermic individuals". *Reprod. Biol. Endocrinol*., 2013, *11*, 101.
[35] Cohlen B.J., Vandekerckhove P., te Velde E.R., Habbema J.D.: "Timed intercourse versus intra-uterine insemination with or without ovarian hyperstimulation for subfertility in men". *Cochrane Database Syst. Rev*., 2000, *2*, CD000360.
[36] Bensdorp A.J., Cohlen B.J., Heineman M.J., Vandekerckhove P.: "Intrauterine insemination for male subfertility". *Cochrane Database Syst. Rev*., 2007, *3*, CD000360.
[37] Van Rumste M.M., Custers I.M., Van der Veen F., van Wely M., Evers J.L., Mol B.W.: "The influence of the number of the number of follicles on pregnancy rates in intrauterine insemination with ovarian stimulation: a meta-analysis". *Hum. Reprod. Update*, 2008, *14*, 563.
[38] Badawy A., Elnashar A., Eltotongy M.: "Effect of sperm morphology and number on success of intrauterine insemination". *Fertil. Steril*., 2009, *91*, 777.
[39] Dorjpurev U., Kuwahara A., Yano Y., Taniguchi T., Yamamato Y., Suto A., *et al.*: "Effect of semen characteristics on pregnancy rate following intrauterine insemination". *J. Med. Invest*. 2011, *58*, 127.
[40] Berker B., Sukur Y.E., Kahraman K., Atabekoglu C.S., Sonmezer M., Ozmen B., Ates C.: "Absens of rapid and linear progressive motile spermatozoa 'grade A' in semen specimens: does it change intrauterine insemination outcomes?". *Urology*, 2012, *80*, 1262.
[41] Van Weert J.M., Repping S., Van Voorhis B.J., Van der Veen F., Bossuyt P.M., Mol B.W.: "Performance of the postwash total motile sperm count as a predictor of pregnancy at the time of intrauterine insemination: a meta-analysis". *Fertil. Steril*., 2004, *82*, 612.
[42] Guven S., Gunalp G.S., Tekin Y.: "Factors influencing pregnancy rates in intrauterine insemination cycles". *J. Reprod. Med*., 2008, *53*, 257.
[43] Nikbakht R., Saharkhiz N.: "The influence of sperm morphology, total motile sperm count of semen and the number of motile sperm inseminated in sperm samples on the success of intrauterine insemination". *Int. J. Fertil. Steril*., 2011, *5*, 168.
[44] Ombelet W., Dhont N., Thijssen A., Bosmans E., Kruger T.: "Semen quality and prediction of IUI success in male subfertility: a systematic review". *Reprod. Biomed. Online*, 2014, *28*, 300.
[45] Sakkas D., Manicardi G.C., Tomlinson M., Mandrioli M., Bizzaro D., Bianchi P.G., Bianchi U.: "The use of two density gradient centrifugation techniques and the swim-up method to seperate spermatozoa with chromatin and nuclear DNA anomalies". *Hum. Reprod*., 2000, *15*, 1112.
[46] Morrell J.M., Moffatt O., Sakkas D., Manicardi G.C., Bizzaro D., Tomlinson M., *et al.*: "Reduced senescence and retained nuclear DNA integrity in human spermatozoa prepared by density gradient centrifugation". *J. Assist. Reprod. Genet*., 2004, *21*, 217.
[47] Posada M.N., Azuero A.M., Arango A.M., Raigosa G.C., Cano J.F., Perez A.L.: "Sperm washing with swim-up versus gradients in intra uterine insemination (IUI): results of a prospective randomised study comparing pregnancy rates and costs". 61st ASRM meeting. *Fertility and Sterility Abstract Book*, 2005, *84 Suppl 1*, 361.
[48] Carrel D.T., Kuneck P.H., Peterson C.M., Hatasaka H.H., Jones K.P., Campbell B.F.: "A randomized, prospective analysis of five sperm preparation techniques before intrauterine insemination of husband sperm". *Fertil. Steril*., 1998, *69*, 122.
[49] Dudson W.C., Moessner J., Miller J., Legro R.S., Gnatuk C.L.: "A randomized comparison of the methods of sperm preparation for intrauterine insemination". *Fertil. Steril*., 1998, *70*, 574.

Corresponding Author:
S. FILIZ, M.D.
Kocaeli University Umuttepe Campus
Faculty of Medicine, IUF Center
Kocaeli 41380 (Turkey)
e-mail: serdarfiliz@yahoo.com