ANALGESIA WITH REMIFENTANIL VERSUS ANESTHESIA WITH PROPOFOL-ALFENTANIL FOR TRANSVAGINAL OOCYTE RETRIEVAL: A RANDOMIZED TRIAL ON THEIR IMPACT ON *IN VITRO* FERTILIZATION OUTCOME

Paraskevi Matsota*, Tatiana Sidiropoulou*, Chrysanthi Batistaki*, Dimitrios Giannaris**, Aggeliki Pandazi*, Helen Krepi*, Kalliopi Christodoulaki* and Georgia Kostopanagiotou*

Abstract

Background: The aim of this study was to compare the effects of analgesia with remifentanil versus anesthesia with propofol and alfentanil on in vitro fertilization outcome.

Methods: The study included 58 women undergoing ultrasound transvaginal oocyte retrieval, who were randomized to receive either analgesia with remifentanil (n=29) or anesthesia with propofol and alfentanil (n=29)**.** The subjects were compared for number of collected and matured oocytes, fertilization rate, cleavage rate, implantation rate, pregnancy rate, and embryo quality. Anesthesia related side effects and both patient and gynecologist satisfaction were recorded.

Results: There were no significant differences in collected oocytes, matured oocytes, fertilization and cleavage rate, embryo quality and implantation and pregnancy rate between the two groups. There was no difference regarding side effects and both patient and gynecologist satisfaction.

Conclusions: Analgesia with remifentanil compared with anesthesia with propofol and alfentanil, provided equally effective and safe anesthesia during ultrasound transvaginal oocyte retrieval.

Introduction

Ultrasound transvaginal oocyte retrieval constitutes a day case surgery and can be performed under different patterns of anesthesia such as sedation, and general or locoregional anesthesia[1-3]. However, the administered anesthetic agents, opiates and local anesthetics have been detected in the follicular fluid[4-6], and their deleterious potentials on the oocytes have been reported in experimental studies[7,8]. In human beings, previous studies have investigated the effect of anesthesia on reproductive techniques outcome but have yielded contradictory findings[9-12]. Differences with regard to the study design and randomization, the anesthetic drugs used, or the anesthetic

* 2nd Department of Anesthesiology, School of Medicine, University of Athens, "Attikon" Hospital, Athens, Greece.

** 3nd Department of Obstetrics and Gynecology, School of Medicine, University of Athens, "Attikon" Hospital, Athens, Greece.

Corresponding Author: Paraskevi Matsota, Assistant Professor of Anesthesiology, School of Medicine, University of Athens, "Attikon" Hospital, Athens, Greece. 1 Rimini Str, Chaidari, Postal Code 12462, Athens, Greece, Phone + + 30-210-5015827, FAX + +30-210-5326413, e-mail: matsota@yahoo.gr

technique performed, may are attributed to these different findings. Thus, we re-examined the question of whether anesthetic regimen used during ultrasound transvaginal oocyte retrieval is associated with in vitro fertilization (IVF) outcome using a standard research protocol.

Propofol, remifentanil, and alfentanil are indicated for anesthesia provision in day case operations due to their rapid onset of action and short duration. In the present prospective randomized study, our primary goal was to compare the effect of analgesia with remifentanil versus anesthesia with propofol and alfentanil on IVF outcome in terms of their impact on fertilization rate, cleavage rate, implantation rate, pregnancy rate, and embryo quality. A secondary aim was to compare their anesthetic profile with regard to their efficacy, safety and acceptance of both patient and gynecologist.

Materials and Methods

The clinical trial took place at the "ATTIKON" University Hospital (Athens, Greece) after the study protocol was approved by the Hospital Ethics and Research Committee (Chairperson Prof S. Raptis) on 18 February 2004. The study was a prospective randomized comparative study and performed in accordance with good clinical practice guidelines. All oocyte retrieval procedures were performed by the same gynecologist. Written informed consent was obtained from all women.

Patients

Fifty eight women, ASA I-II, scheduled for ultrasound transvaginal oocyte retrieval as day-case patient, were enrolled in the present study. Ovarian stimulation was performed with either gonadotrophin releasing hormone (GnRH) analogue (protocol I) or GnRH antagonist (protocol II) and followed by recombinant follicular stimulating hormone (rFSH). Human chorionic gonadotrophin (HCG) was administered when at least three follicles attained a mean diameter of 17 mm. Ultrasound transvaginal oocyte retrieval was performed 36-38 hours after HCG administration. The prognostic factors as age, body weight, duration of anesthesia, smoking habit, infertility (primary or secondary) and IVF protocol were recorded.

Exclusion criteria

Exclusion criteria in terms of anesthesia included: patient refusal, morbid obesity, increased gastroesophagic reflux; history of severe cardiovascular, respiratory or other systemic disease; known history of allergy or sensitivity to any anesthetic drug used in the present study.

In terms of infertility history the exclusion criteria included: women's age >44 years; primary ovarian failure and basal FSH>12 IU/L; women to be known as poor responders in previous IVF cycles (less than 3 oocytes obtained); women with more than 3 previous attempts.

Study design

All patients were fasted and unpremedicated, and received midazolam 2 mg intravenous (iv) just before starting the procedure. Afterwards, women were randomly assigned into two groups (with the closed envelope method, 29 women each group) and received either analgesia with remifentanil (Ultiva™ solution 50 $\mu g.ml^{-1}$) (REM group) or anesthesia with propofol and alfentanil (PA group).

In REM group, a bolus dose 1 $\mu g.kg^{-1}$ of remifentanil was administered slowly during one minute following by a continuous iv infusion in a rate of 0.15-0.4 $\mu g.kg^{-1}.min^{-1}$. In PA group, induction of anesthesia was achieved with iv propofol 2 $mg.kg^{-1}$ and alfentanil 15 $\mu g.kg^{-1}$, and was maintained with propofol continuous infusion in a rate of 2-4 $mg.kg^{-1}.h^{-1}$. The continuous infusion of either remifentanil or propofol was stopped after the extraction of the last oocyte, while the total dose given was recorded, respectively. All patients were spontaneously breathing. A Venturi mask was used to provide a 50% oxygen-enriched air. Noninvasive blood pressure (NIBP), ECG, SpO_2 *(Datex-Ohmeda, 5250 RGM, Louisville, USA)*, and end tidal PCO_2 were included in the intraoperative monitoring.

Intraoperatively, adverse effects such as airway obstruction and rigidity, as well as the necessity for ventilation support using the bag-mask technique (whenever an increase of end-tidal PCO_2 >40 mmHg

or a decrease of SpO_2 <97% was observed) were recorded. Moreover, intra- and/or postoperative nausea and vomiting was estimated using a 4-scale descriptive scale (none, nausea only, less than 2 episodes of vomitus, more than 2 episodes of vomitus). Duration of anesthesia, total number of collected oocytes, and number of matured oocytes were also recorded. Postoperatively, both patient and gynecologist satisfaction regarding anesthetic technique was assessed by a 2-point scale (yes/no) for the following questions: Are you satisfied with the anesthetic technique used? Would you prefer again the same anesthetic?

Laboratory Fertilization Procedures

During oocyte retrieval procedure, the embryologist (who was blinded to the group allocation) examined the follicular aspirates under a stereo dissecting microscope with heated stage at approximately 37°C and collected the oocytes inside a 4 well culture dish filled with HEPES Buffered MHTF (Modified Human Tubal Fluid) covered with sterile mineral oil. As soon as the oocyte retrieval procedure was completed, the oocytes recovered were washed and transferred in pre-equilibrated culture medium (IVF Fertilizing medium) inside a 4 well culture dish. The name of the patient and the precise oocyte number were clearly written on top of the dish. Then the embryologist returned the specific dish inside the incubator, where it remained until the time of insemination (approximately 3-4 hours after oocyte retrieval procedure or 40 hours after ovulation induction). The interval between the induction of sedation and transfer of retrieved oocytes into the incubator and culture medium was less than 15 minutes.

The incubators in IVF are using 6% CO_2 gas or gas mixture, in order to maintain stable pH (7.2-7.4) in the culture medium and temperature (37° C).

After insemination the embryologist returned the oocytes inside the incubator where they were remaining overnight. The next morning (16-20 hours after insemination), fertilization was assessed under an inverted microscope and normal fertilized oocytes were transferred in a new culture dish with fresh pre-equilibrated culture medium (cleavage medium) and returned inside the incubator where they were remaining until the day of transfer (usually day-2 or day-3 after oocytes retrieval procedure).

Oocytes were fertilized either via the conventional insemination (IVF) or the intracytoplasmic sperm injection (ICSI) based on the couples' fertility history. ICSI was performed in cases with male factor or previous failure of fertilization in standard IVF, in some cases of unexplained infertility as well. Fertilization was assessed 16-18 hours after conventional insemination or ICSI procedure. Embryo transfer was performed either on day 2 or day 3 after the oocyte retrieval. Up to 3 embryos were transferred according to the couples' clinical features. Good quality embryos were defined as grade-1 and grade-2 according to the morphological features following the scale 1-5 (grade-1: the best, grade-5: the worst)[13]. IVF parameters under investigation were: Fertilization rate: number of oocytes fertilized to the number of oocytes obtained;

Cleavage rate: number of embryos divided to the total number of embryos; Embryo quality: the number of good quality embryos meaning the total number of grade-1 and grade-2 embryos;

Frozen embryos: The number of grades 1 and 2 embryos were cryostored. According to the protocol only good embryos (grade 1 and 2) are cryostored; Implantation rate: number of gestational sacs to the number of embryos transferred;

Clinical pregnancy rate: no of ongoing pregnancy>16 weeks of gestation/ total women who underwent IVF treatment per transferred.

Statistical Analysis

Based on data obtained from our centre we calculated that approximately 66.67% of the total number of oocytes would be fertilized. We estimated that a 25% difference on the fertilization rate of oocytes between the two groups ($\alpha = 0.05$; $1-\beta = 80\%$) would be clinically significant and therefore a minimum of 135 oocytes would be required per group. Given our sample characteristics (poor IVF responders and women with more than 3 previous attempts would be excluded) we calculated from our centre statistics that approximately 5 oocytes would be retrieved as a mean from each woman. We therefore decided to enroll at

least 28 women per group to reach adequate power.

Statistical analyses were performed with SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA). Results in text and tables are expressed as mean ± SD, number/percentage within group or median (range) as appropriate. Normally distributed data were analyzed using the Student's T test or analysis of variance for repeated measurements with the Bonferroni correction, whereas for analysis of categorical and skewed data Mann – Whitney U test, χ^2 test, or Kruscal-Wallis tests were used as appropriate. Regression analysis was used in order to assess impact of independent variables. A value of $P < 0.05$ was considered statistically significant.

Results

Fifty eight women were consecutively enrolled in the present study (Figure 1). Demographics and prognostic factors are listed in table 1. No significant differences in age, body weight, smoking habit, ASA physical status, cause of infertility, ovulation protocol used and duration of anesthesia were observed. In all patients the recorded changes of heart rate and NIBP during the observation period where without any clinical importance, as these changes were less than 20% of the baseline values. Remifentanil consumption was 485 ± 215 μg in the REM group and the average infusion rate was 0.334 μg/kg/min. Propofol consumption was 188 ± 73 mg in the PA group and the infusion rate averaged 2.97 mg/kg/hr.

Fig. 1

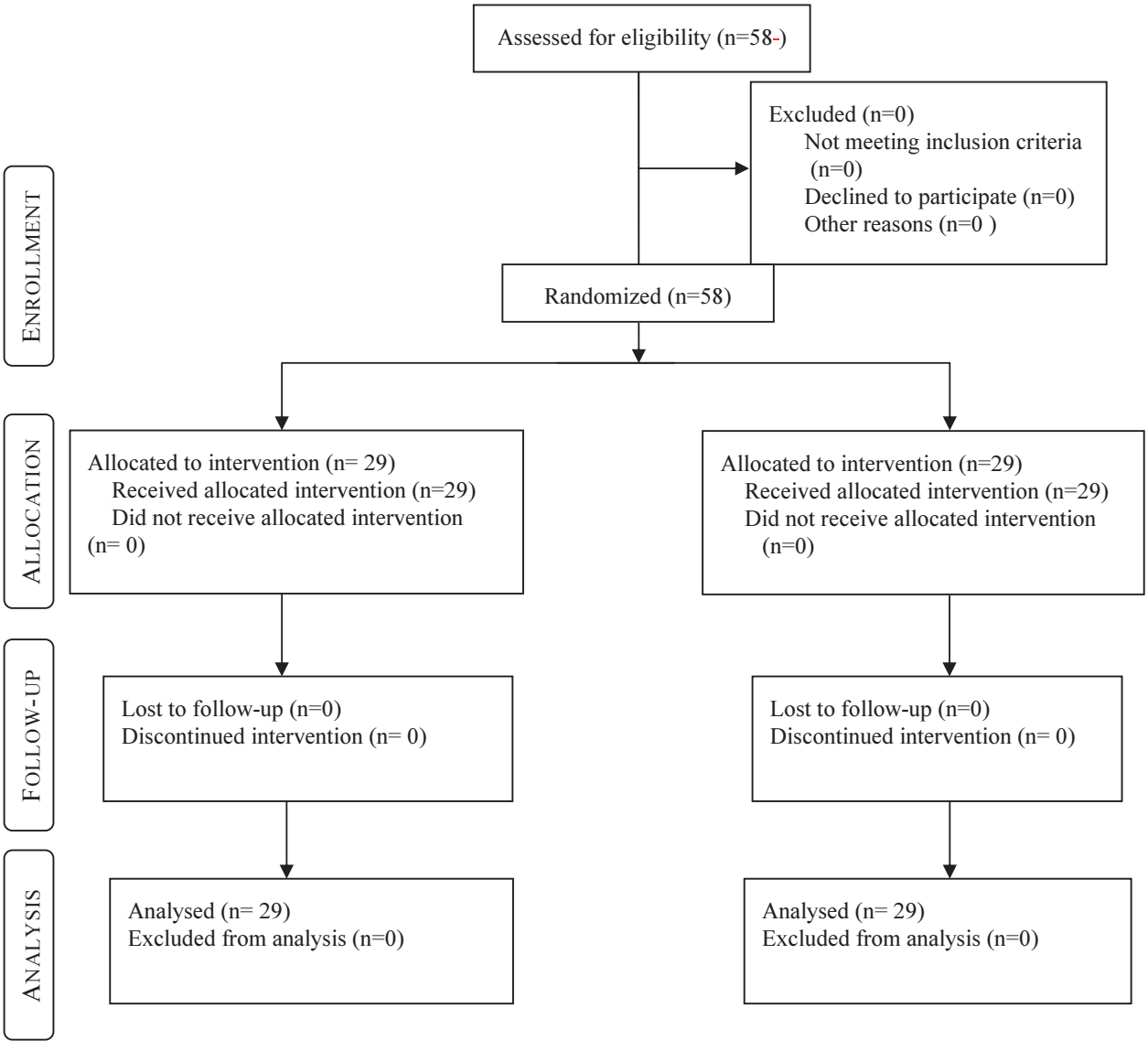

Table 1
Patient demographics, cause of infertility, ovarian stimulation protocol used and duration of anesthesia in the two groups.

	PA n=29	**REM n=29**	***p***
Age (yrs)	35.5 ± 5	34 ± 4.8	0.245
Weight (kg)	61.5 ± 7	62 ± 10	0.669
Smokers (n/%)	9/31%	8/28%	0.773
ASA class (I/II)	28/1	27/2	0.553
Cause of Infertility (Primitive/Secondary)	24/5	27/2	0.227
Ovarian stimulation protocol (I/II)*	13/16	11/18	0.594
Anaesthesia time (min)	21.4 ± 8	20.4 ± 7	0.646

* Protocol I: ovarian stimulation with gonadotrophin releasing hormone analogue.
Protocol II: ovarian stimulation with gonadotrophin releasing hormone antagonist.
PA = propofol-alfentanil group, REM = remifentanil group.

The side effects recorded in both groups (table 2) were similar with the exception of airway obstruction and need for bag ventilation that was significantly higher in the PA group. Though no difference was observed regarding nausea and vomiting, two patients in propofol-alfentanil group had 1 episode of vomiting postoperatively. Patient satisfaction was similar in both groups (PA=29 patients, REM=27 patients) and the vast majority of patients were willing to repeat the procedure with the same anesthesia protocol (PA=29 patients, REM=27 patients). Similarly, surgeon satisfaction was similar in both groups and only in one patient per group the surgeon was not satisfied with the anesthesia protocol.

Table 2
Incidence of side effects in the two groups

	PA n=29	**REM n=29**	***P***
Nausea and vomiting:			
- None	27	29	
- Nausea	0	0	0.150
- < 2 episodes of vomitus	2	0	
- > 2 episodes of vomitus	0	0	
Airway Obstruction	6	1	0.044
Need for Mask Ventilation	23	5	0.001
Rigidity	0	1	0.313

PA = propofol-alfentanil group, REM = remifentanil group.

The number of oocytes retrieved, fertilized as well as the number of embryos cleaved and transferred are summarized in table 3. We also report the mean number of frozen embryos in each group. A post hoc power analysis revealed that from the data obtained from our study, power was calculated 87% for cleavage rate and 89% for the implantation rate. The results did not differ significantly in the two groups. On the contrary we observed a difference in the cleavage characteristics according to their morphological criteria. More grade-1 embryos were recorded in the REM group whereas more grade-3 embryos were recorded in the PA group and this finding was statistically significant. However when these results were adjusted for age using regression analysis the difference observed in embryo quality was no longer statistically significant. No other significant differences were observed in the implantation rate or the number of embryos that achieved pregnancy (table 3).

Table 3
Effect of anesthetic technique on in vitro fertilization outcome

	PA	**REM**	***P***
Women	29	29	-
Oocytes retrieved (n)	230	271	-
Mature oocytes (n / %)	155 / 67%	199 / 73%	NS
Oocytes fertilized (n/%)	146 / 63.5%	165 / 60.8%	NS
Embryos cleaved (n/%)	112 / 76.7%	129 / 78.2%	NS
Embryos transferred (n)	59	58	
- Quality 1 embryos (n) - Quality 2 embryos (n) - Quality 3 embryos (n)	28 23 8	36 21 1	0.0382
Embryos cryostored (mean ±SD)	1.4 ± 2.3	2.5 ± 3.8	NS
Embryos implanted (n/%)	12 / 20.3%	15 /25.8%	NS
Clinical pregnancy rate	10/29 (34.4%)	10/29 (34.4%)	NS

PA = propofol-alfentanil group, REM = remifentanil group. NS = not significant.

Discussion

In the present study, we investigated comparatively two different anesthetic techniques performed to women undergoing ultrasound transvaginal oocyte

retrieval: analgesia with remifentanil versus anesthesia with propofol and alfentanil. We tested the hypothesis of whether these two different anesthetic regimens could affect differently the IVF outcome and we found no differences between them with respect to the number of collected and matured oocytes, and the fertilization, cleavage, implantation and pregnancy rate, respectively. The observed pregnancy rate in both groups was similar and was in agreement with the pertinent literature on IVF achieved pregnancy rate[14]. On the contrary, the superiority of remifentanil was revealed initially with regard to the quality of embryo transferred. However, this difference failed to hold true when our results were adjusted for age. Noticeably, no difference was observed in the number of the frozen embryos, and we have to point out that the frozen embryos were only good quality, since according to our protocol only good quality embryos (grade-1 and grade-2) were cryostored. The embryo quality is strongly related to the implantation potential, that means the likelihood of implantation for grade-1 and grade-2 embryos is higher compared to grade 3 and 4[15]. So far, no differences in terms of embryo quality have been described between the two different protocols used for ovarian stimulation[16], but it is well known that IVF success is multifactorial hence a multiplicity of other variables may play a role.

Oocyte retrieval is a short but quite stressful experience for women. Social and psychological factors contribute to the stress**,** while pain results from penetration of the vaginal mucosa and the ovarian capsule. In the present study, midazolam was given to all patients for allaying their anxiety, since midazolam has not been proven having detrimental effects on IVF outcome**,** although small amounts of this benzodiazepine can be detected in follicular fluid[4,17].

For analgesia provision during the entire procedure we administered either alfentanil or remifentanil. Both opioids are indicated for day-case procedures due to their pharmacokinetic profile. Alfentanil has been shown to have a very low penetration into the follicular fluid achieving concentrations about ten-fold smaller than those in the serum at the same time points[5]. Continuous intravenous administration of remifentanil for oocyte retrieval was compared with local anesthesia in a recent study by Milanini et al, who found that remifentanil facilitates the retrieval of oocytes without interfering in their quality or embryo score[3]. Moreover, Hammadeh et al, recommend the use of remifentanil for IVF oocyte retrieval, since they did not find any negative effect on IVF outcome, when they compared the combination of remifentanil with either propofol or isoflurane versus sedation with midazolam or propofol[18]. Of interest, their cleavage and pregnancy success rates (namely, 53.3% and 23.6%, respectively) were lower than our findings in those patients who received remifentanil (78.2% and 25.8%, respectively). These differences could be attributed to the fact that in their study remifentanil was not used as a sole anesthetic agent but was administered in combination with either propofol or isoflurane.

Propofol is widely used in assisted reproduction but its effect on IVF outcome has not been completely clarified. Though Christiaens et al[6], have shown that propofol follicular concentration increases with time, more recent studies have not documented a determinant effect on reproductive outcome[9,11]. In a comparison between propofol-based general anesthesia and paracervical block, no difference has been found between the fertilization rates or embryo cleavage characteristics[12]. Conversely, Coetsier et al, report a time-depended toxic effect of propofol even at very low concentrations depending on the duration of the exposure, but a small number of patients have been included in their study[19].

In a retrospective study by Wilhelm et al, patients who received analgesia with remifentanil had a significantly greater pregnancy rate with IVF (28.2%) comparing to those underwent general anesthesia with either propofol or isoflurane and alfentanil (16.3%)[20]. Three reasons may have contributed to the decreased pregnancy rate observed in their work in those patients who underwent general anesthesia. First, the propofol maintenance rate used in the above study (4-8 $mg.kg^{-1}.h^{-1}$) was two-fold higher comparing to our study (propofol continuous infusion 2-4 $mg.kg^{-1}.h^{-1}$). Second, in their study the duration of anesthesia was significantly longer with general anesthesia than with remifentanil analgesia (namely, 50±12 vs 28±8, respectively), a fact with increased clinical significance as prolonged exposure to anesthetics may adversely affect oocyte fertilizability[19]. Third, propofol co-administered

with 60% N_2O in oxygen, a combination which has been proven to have deleterious effect on oocytes and cleavage ratio[21]. In our prospective randomized study, a 50% oxygen-enriched air was provided to all patients, while no difference was revealed between the two groups regarding the duration of anesthesia (mean time was 20.4 and 21.4 in remifentanil and propofol-alfentanyl group, respectively), which was almost 2.5 fold shorter than the mean time observed in the general anesthesia group of the above study.

The optimal anesthetic technique for IVF should provide increased comfort level of both patient and gynaecologist to maximize the harvesting of oocytes, and have no side effects. Therefore, we compared the anesthetic profile of analgesia with remifentanil versus general anesthesia with propofol and alfentanil. In the present study, no differences were revealed between the two under investigation anesthetic techniques regarding the number of collected oocytes, side effects (with the exception of the observed increased airway obstruction and need for bag-mask ventilation in propofol-alfentanyl group), and satisfaction. Both patients and gynecologist reported increased satisfaction, a fact that is really important, since there is lack of studies regarding patient and/or gynecologist satisfaction, especially with anesthesia performed for reproductive procedures.

In conclusion, the clinical suitability for ultrasound transvaginal oocyte retrieval of both analgesia with remifentanil and anesthesia with propofol - alfentanil has been shown in our study. Both anesthetic regimens revealed similar anesthetic profile with regard to their efficacy, safety, and satisfaction of both women and gynecologist, and affected similarly fertilization, cleavage, embryo quality, implantation, and pregnancy rate. However, the present study was limited to embryo quality test and not to the whole pregnancy process and neonate development, thus further investigation is needed to confirm and extend our findings.

References

1. Jain D, Kohli A, Gupta L, Bhadoria P, Anand R: Anaesthesia for In Vitro Fertilisation. *Indian J Anaesth;* 2009, 53:408-413.
2. Botta G, D'Angelo A, D'Ari G, Merlino G, Chapman M, Grudzinskas G: Epidural anesthesia in an in vitro fertilization and embryo transfer program. *J Assist Reprod Genet;* 1995, 12:187-90.
3. Milanini MN, D'Onofrio P, Melani Novelli AM, Dabizzi S, Cozzi C, Evangelisti P, Quttadamo L, Noci I: Local anesthesia versus intravenous infusion of remifentanil for assisted reproductive technologies. A retrospective study. *Minerva Ginecol;* 2008, 60:203-7.
4. Chopineau J, Bazin JE, Terrisse MP, Sautou V, Janny L, Shoeffler P, Bastide P: Assay for midazolam in liquor folliculai during in vitro fertilization under anaesthesia. *Clin Pharm;* 1993, 12:770-3.
5. Shapira SC, Chrubasik S, Hoffmann A, Laufer N, Lewin A, Magora F: Use of alfentanil for in vitro fertilization oocyte retrieval. *J Clin Anesth;* 1996, 8:282-5.
6. Christiaens F, Janssenswillen C, Verborgh C, Moerman E, Devroey P, Van Steirteghem A, Camu F: Propofol concentrations in follicular fluid during general anaesthesia for transvaginal oocyte retrieval. *Hum Reprod;* 1999, 14:345-348.
7. Alsalili M, Thornton S, Fleming S: The effect of the anaesthetic, Propofol, on in-vitro oocyte maturation, fertilization and cleavage in mice. *Hum Reprod;* 1997, 12:1271-4.
8. Janssenswillen C, Christiaens F, Camu F, Van Steirteghem A. The effect of propofol on parthenogenetic activation, in vitro fertilization and early development of mouse oocytes. *Fertil Steril;* 1997, 67:769-74.
9. Huang HW, Huang FJ, Kung FT, Tsai MY, Lin H, Chang sy, Hsu YH, Chang hw: Effects of induction anesthetic agents on outcome of assisted reproductive technology: a comparison of propofol and thiopental sodium. *Chang Gung Med J;* 2000, 23:513-9.
10. Palot M, Harika G, Visseaux H, Guillet H, Pigeon F, Leon A, Rendoing J: Use of nitric oxide in general anaesthesia for oocyte retrieval. *Ann Fr Anesth Reanim;* 1989, 8:R147.
11. Ben-Shlomo I, Moskovich R, Golan J, Eyali V, Tabak A, Shalev E: The effect of propofol anesthesia on oocyte fertilization and early embryo quality. *Hum Reprod;* 2000, 15:2197-9.
12. Christiaens F, Janssenswillen C, Van Steirteghem AC, Devroey P, Verborgh C, Camu F: Comparison of assisted reproductive technology performance after oocyte retrieval under general anaesthesia (propofol) versus paracervical local anaesthetic block: a case-controlled study. *Hum Reprod;* 1998, 13:2456-60.
13. Elder K, Dale B: Embryo quality and selection for transfer. In: Elder K, Dale B (editors): *In vitro fertilization* (2nd ed). Cambridge: Cambridge University Press; 2000, pp. 167-74.
14. Trout SW, Vallerand AH, Kemmann E: Conscious sedation for in vitro fertilization used in paracervical block during oocyte retrieval. *J Assist Reprod Genet;* 2003, 20:8-12.
15. Cohen J, Inge KL, Suzman M, Wiker SR, Wright G: Videocinematography of fresh and cryopreserved embryos: a retrospective analysis of embryonic morphology and implantation. *Fertil Steril;* 1989, 51:820-7.
16. Kolibianakis EM, Collins J, Tarlatzis BC, Devroey P, Diedrich K, Griesinger G: Among patients treated for IVF with gonadotrophins and GnRH analogues, is the probability of live birth dependent on the type of analogue used? A systematic review and meta-analysis. *Hum Reprod Update;* 2006, 12:651-71.
17. Swanson R, Leavitt M: Fertilization and mouse embryo development in the presence of midazolam. *Anesth Analg;* 1992, 74:549-54.
18. Hammadeh ME, Wilhelm W, Huppert A, Rosenbaum P, Schmidt W: Effects of general anaesthesia vs. sedation on fertilization, cleavage and pregnancy rates in an IVF program. *Arch Gynecol Obstet;* 1999, 263:56-9.
19. Coetsier T, Dhont M, De Sutter P, Merchiers E, Versichelen L, Rosseel MT. Propofol anaesthesia for ultrasound guided oocyte retrieval: accumulation of the anaesthetic agent in follicular fluid. *Hum Reprod;* 1992, 7:1422-4.
20. Wilhelm W, Hammadeh ME, White PF, Georg T, Fleser R, Biedler A: General anesthesia versus monitored anesthesia care with remifentanil for assisted reproductive technologies: effect on pregnancy rate. *J Clin Anesth;* 2002, 14:1-5.
21. Palot M, Visseaux H, Harika G: Effect of nitrous oxide and/or halothane on cleavage rate during general anaesthesia for oocyte retrieval. *Anesthesiology;* 1990, 73:A930.