The effects of general anaesthesia with sevoflurane on intracytoplasmic sperm injection outcomes

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RESUMO
Objetivos: investigar os potenciais efeitos adversos da anestesia geral, utilizada para recuperação oocitária, nos resultados de injeção intracitoplasmática de espermatozoides (ICSI). Material e métodos: Um total de 444 ciclos de ICSI, em que uma combinação de fentanilo, propofol e sevoflurano foi utilizada para anestesia geral durante a aspiração oocitária, foram avaliados. Modelos de regressão foram utilizados para avaliar a influência do tempo de período médio de anestesia por fólio aspirado (ΔT) nos resultados da ICSI. Os ciclos foram divididos em grupos de acordo com o ΔT, e os resultados da ICSI foram comparados entre os grupos. Resultados: analise de regressão demonstrou uma correlação negativa entre o ΔT e a taxa de embriões de boa qualidade (Slope: -5.015; R²: 4.4%; p = 0.001). O ΔT foi determinante para a diminuição da probabilidade de gravidez (OR: 0.77; IC 95%: 0.64-0.93; p = 0.005) e transferência de embriões (OR: 0.17; IC 95%: 0.06-0.48; p = 0.001). Quando os ciclos foram divididos de acordo com o ΔT, diferenças significativas nas taxas de embriões de boa qualidade (ΔT: 55.6% vs 44.2%; p = 0.001) e de gestação (ΔT: 58.2% vs 41.6%; p = 0.045) foram observadas para valores de ΔT ≤ 2 e > 2 min, respectivamente. Conclusão: nosso estudo sugere que a anestesia geral influencia negativamente o desenvolvimento do embrião e as taxas de geração e de cancelamento de ciclo. Palavras-chave: ICSI; recuperação oocitária; anestesia.

ABSTRACT
Objective: to investigate the potential adverse effects of general anaesthesia, used for oocyte retrieval, in intracytoplasmic sperm injection (ICSI) outcomes. Material and methods: A total of 444 ICSI cycles in which a combination of fentanyl, propofol and sevoflurane was used for general anaesthesia during follicle aspiration were evaluated. Regression models were carried out to assess the influence of the mean period of anaesthesia per aspirated follicle (ΔT) on ICSI outcomes. In a further analysis, the cycles were split into groups according to the ΔT, and ICSI outcomes were compared between the groups. Results: regression analysis demonstrated a negative correlation between ΔT and the percentage of high-quality embryos (Slope: -5.015; R²: 4.4%; p < 0.001). The ΔT was determinant to the decreased likelihood of pregnancy (OR: 0.77; CI 95%: 0.64-0.93; p = 0.001) and embryo transfer (OR: 0.17; CI 95%: 0.06-0.48; p = 0.001). When the cycles were split according to ΔT, significant differences in the high-quality embryos rate (55.6% vs 44.2%; p = 0.001) and the pregnancy rate (58.2% vs 41.6%; p = 0.045) were observed for values of ΔT ≤ 2 and > 2 min, respectively. Conclusion: Our study suggests that the general anaesthesia negatively influences embryo development, pregnancy rate and cycle cancellation rate.

Keywords: ICSI; oocyte retrieval; anaesthesia.

INTRODUCTION
Since 1976, when the first successful in vitro fertilization (IVF) occurred (Steinbock and Edwards, 1978), there has been a constant increase in the use of IVF in couples with un treatable infertility. The IVF treatment requires the harvesting of oocytes from the patients’ ovaries. Initially, oocyte retrieval was performed by laparoscopy; through refinements in ultrasound technologies, however, oocyte retrieval has progressed from laparoscopic to transvaginal aspiration. Transvaginal follicular aspiration is generally performed with the administration of anaesthesia, such as conscious sedation, neuraxial anaesthesia, general anaesthesia, injection of local anaesthetic agents, or any combination of the above (Vlahos et al., 2009). The optimal choice for assisted reproductive technology ART anaesthesia is controversial; in part because of the unclear impact anaesthesia may have on IVF outcomes.

Any anaesthetic technique may potentially interfere with certain aspects of human oocyte fertilization or embryo implantation (Van de Veide et al., 2005). Because the majority of anaesthetic agents have been detected in follicular fluid within minutes of their administration (Imme- dienne et al., 1992; Soussis et al., 1995; Wikland et al., 1990), an increased awareness of the potential adverse effects of different types of anaesthesia on the quality of the oocytes, which may eventually interfere with embryo development and pregnancy success, has developed. Different anaesthetic doses, combinations, and exposure durations may potentially affect IVF results. Indeed, the duration of exposure to anaesthetic agents appears to play an important role, as observed by Boyers et al. (Boyers et al., 1997), who compared the first and last oocytes collected under lapa roscopy under general anaesthesia and reported that the last oocyte uniformly had lower fertilization rates.

General anaesthesia, which is defined as a depressed level of consciousness, can be accomplished by inhalational agents (Vlahos et al., 2009), which represent basic drugs used in modern balanced anaesthesia (Totit). Halogenated compounds are inhalational agents, which
have been hypothesized to increase cytoplasmic cleavage
alterations (Yen, 2007) and mitotic abnormalities (Kusyk
and Hsu, 1976), and to reduce reproductive success in
clinical practice (Wilhelm et al., 2002). Sevoflurane is an
ether inhalation general anaesthetic agent (Hassel and Gaa,
1996) that provides rapid anesthetic induction and emerg-
ence from anaesthesia, compared to other inhalational
anaesthetic agents (Kanazawa et al., 1999).

Several studies have evaluated the effects of general
anaesthesia on IVF outcome (Christiasen et al., 1998;
Christchow et al., 1991; Fisher et al., 1987; Hammadah et
al., 1999; Wilhelm et al., 2002). However, the effects of
general anaesthesia with sevoflurane on IVF outcomes
have never been investigated. The purpose of this study
was to investigate the potential adverse effects of general
anaesthesia with sevoflurane used for oocyte retrieval, in
intracytoplasmic sperm injection (ICSI) outcome.

MATERIAL AND METHODS

Experimental design

Using our center’s computerized database, we retro-
spectively identified 444 women who, from July 2009 to March
2010, underwent ultrasound-guided follicular aspiration,
under general anaesthesia through halogenated agents,
for an ICSI cycle.

In order to avoid bias, the following ICSI cycles were
excluded from the study: (i) cycles in which six or less
oocytes were retrieved, because, generally, the duration
of ovarian puncture increases in the presence of fewer
follicles; (ii) cycles performed in women over 35 years
of age, because these patients present diminished ovar-
ian response to gonadotrophins; (iii) cycles combined
with preimplantation genetic diagnosis (PGD), and (iv)
cycles which used non-oocytes.

Written informed consent, in which the patients agreed
to share the outcomes of their cycles for research purposes,
was obtained. The study was approved by the local Insti-
tutional Review Board.

Controlled ovarian stimulation

Controlled ovarian stimulation was achieved by long pit-
uitary down regulation using a gonadotrophin-releasing
hormone agonist (GnRH agonist, Lupron®; Serono, Gene-
vie, Switzerland). Oocyte retrieval was performed 30 hours
after the administration of recombinant human chorionic
gonadotrophin (hCG; Ovidrel®, Serono, Gennevilliers,
France) through transvaginal ultrasonography.

Anesthetic Procedure

All patients had fasted for at least eight hours. For general
anaesthesia, the patients received anaesthetic induction with
fentanyl (1.3 μg/kg) and propofol (2 mg/kg). For mainte-
nance, patients received sevoflurane and 100% oxygen. At
the end of the procedure, the patients received ondanset-
ron 4 milligrams as prophyaxis for nausea and vomiting,
and dipiron 20-30 mg/kg for postoperative analgesia.

Intracytoplasmic sperm injection and as-
essment of fertilization

Oocytes were transferred into a micro-injection dish,
prepared with 4 μL drops of buffered medium (HEPES;
Irvine Scientific, Santa Ana, USA), and covered with
mineral oil on a heated stage at 37.0 ± 0.5°C of an
inverted microscope. Approximately 15 minutes after ICSI,
fertilization was confirmed by the presence of two pronu-
dlei and the extrusion of the second polar body.

Embryo quality and embryo transfer

Embryos were kept in a 50 μL drop of HTF medium
supplemented with 10% HSA covered with mineral oil in
a humidified atmosphere under 6% CO2 at 37°C until
transfer. Embryonic development was evaluated on days
2 and 3. The following parameters were recorded: (i)
number of blastomeres, (ii) fragmentation percentage,
(iii) variation in blastomere symmetry, (iv) presence of
multinucleation and (v) defects in the zona pellucida and
cytoplasm. High-quality embryos were defined as those
having all of the following characteristics: eight to ten
cells, less than 15% fragmentation, symmetric blasto-
meres, absence of multinucleation, colourless cytoplasm
with moderate granulation and no inclusions, absence of
perivitelline space granularity and absence of zona pellu-
cida dysmorphism. Embryos lacking any of the above
characteristics were considered to be of low quality.
Embryo transfer was performed on day 3 of development.
One to three embryos were transferred per couple.

Clinical Follow-up

A pregnancy test was performed 12 days after embryo
transfer; a positive pregnancy test was considered to
define a biochemical pregnancy. All women with a posi-
tive test had a transvaginal ultrasound scan at 2 weeks
after the positive test; a clinical pregnancy was diag-
nosed when fetal heartbeat was detected. To calculate the
implantation rate, the number of gestational sacs was
divided by the number of embryos transferred. Miscar-
riage was defined as spontaneous abortion before 20
weeks’ gestation.

Statistical analysis

Regression models were carried out to assess the influ-
ence of the mean period of anaesthesia per aspirated
follicle (ΔT) on ICSI outcomes. The results are reported
as slopes and R-squared (R²) or odds ratios (OR), with
their 95% confidence intervals (CI) and p values. The
results were considered to be significant at the 5% criti-
cal level (p < 0.05). In a further analysis, the cycles were
split into groups according to the ΔT: Group 1: < 2
minutes, which included patients with ΔT ≤ 2 minutes,
and Group 2: ≥ 2 minutes, which included patients with
ΔT > 2 minutes. The ICSI outcomes were compared be-
 tween the groups. The results are expressed as means ±
standard deviation (SD) for numeric variables and propor-
tions (%) for categor
cal variables. The results are expressed as odds ratios
(OR), confidence intervals (CI) and p-values and consid-
red to be significant at the 5% critical level (p < 0.05).
Data analysis was carried out using the GraphPad Pri-
oram version 4.0 Statistical Program.

RESULTS

In the first analysis, the cycles’ general characteristics
were as follows: female age = 30.7 ± 5.1 years, total
dose of FSH administered = 1985.6 ± 631.1 IU, oocyte
yield = 71.8 ± 17.8, number of embryos transferred = 2.4 ±
0.9, and mean period of anaesthesia per aspirated
follicle = 2.0 ± 1.2 minutes. ICSI outcomes were as fol-
ows: fertilization rate = 74.3%, percentage of high-quality
embryos = 51.2%, implantation rate = 24.1%, preg-
nancy rate = 44.6% and miscarriage rate = 12.1%. The
regression analysis demonstrated a negative correlation
between ΔT and the percentage of high-quality embryos
(Slope: -0.015; R²: 4.4%; p = 0.001). Moreover, ΔT was
determined to the likelihood of pregnancy (CI: 0.64 – 0.93; p = 0.005) and cycle cancellation (OR:
0.17, CI: 0.06 – 0.46, p = 0.001). No correlations were

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observed between ΔT and the fertilization (Slope: 0.211, R²: 0.012%, p = 0.322), implantation (Slope: 44.291, R²: 0.2%, p = 0.155), or miscarriage rate (OR: 0.87, CI: 0.51 – 1.49, p = 0.604).

In the second analysis, the cycles were split into groups according to ΔT. A total of 226 ICSI cycles were included in groups 2 min. The remaining 183 cycles were included in groups > 2 min. The cycles’ general characteristics were equally distributed between the two groups. Similar female age (30.6 ± 3.2 vs. 31.4 ± 2.8, p = 0.1341), total dose of FSH administered (1952.0 ± 530.6 vs. 1622.3 ± 682.1, p = 0.0634), oocyte yield (71.2 ± 18.5 vs. 70.4 ± 22.3, p = 0.8515), and number of embryos transferred (2.4 ± 0.9 vs. 2.3 ± 0.8, p = 0.4263) were observed between Groups 2 min and Group > 2 min. The comparison of ICSI outcomes between the groups is shown in Table 1.

**DISCUSSION**

Previous reports have suggested that halogenated agents have a negative impact on assisted reproduction outcomes (Cocklow et al., 1991; Fishel et al., 1997; Gonen et al., 1995). Our results suggest that the mean period of general anesthesia per aspirated follicle, with the combination of sevoflurane, fentanyl and propofol, adversely affects ICSI outcome.

Anesthetic agents have been proposed to reach the follicular fluid within minutes after administration, and may have deleterious effects on IVF outcomes (Bailey-Prudhoe et al., 1990; Smoorenburg et al., 1992; Souza et al., 1995; Wickland et al., 1990). Hammadeh et al. (Hammadeh et al., 1999) compared the effects of general anesthesia (with propofol, remifentanyl and sevoflurane) versus sedation (with, or without, propofol) on fertilization rate, cleavage rate and pregnancy rate. The authors reported that despite the fact that general anesthesia seemed to improve the success rate for oocyte retrieval, probably by the improved patient comfort during the transvaginal puncture—the higher number of retrieved oocytes had a lower fertilization rate. Nevertheless, no adverse effects were found in cleavage and pregnancy rates.

Consequently, Wilhem et al. (Wilhelm et al., 2002) observed that pregnancy rates in women undergoing transvaginal oocyte retrieval for assisted reproductive technologies were significantly higher with a remifentanyl-based technique than with a general anesthetic technique. Another study observed similar embryo quality, but reduced pregnancy rates associated with general anesthesia when compared with epidural or paracervical anesthesia (Gonen et al., 1995).

Our study suggests that exposure to general anesthesia is deterministic to the likelihood of pregnancy in ICSI cycles. Indeed, general anesthesia may suppress the secretion of gonadotrophin-releasing hormone and gonadotrophins by the stimulation of prolactin release (Sarker et al., 1976; Sherwood et al., 1980). It has been suggested that the reduction in gonadotrophin levels and the transient elevations of serum prolactin levels have a deleterious effect on the subsequent luteal phase, with the suppression of progesterone production by the corpus luteum (Lehtinen et al., 1987) and endometrial development (Hammadeh et al., 1999). However, in an attempt to evaluate the effect of prolactin levels on IVF, Forman et al. (Forman et al., 1985) observed that elevated plasma or follicular fluid prolactin concentrations have no effects on oocytes or embryo development. Nevertheless, although the difference was not significant, the incidence of pregnancy was highest in the group of patients with the lowest plasma prolactin levels.

Moreover, our results suggest that the higher the period of exposure to general anesthesia, the lower the percentage of high-quality embryos. Additionally, in our study, exposure to general anesthesia was determinant to the likelihood of cycle cancellation, given the non-availability of embryos to be transferred. Indeed, halogenated agents have been proposed to cause cytoplasmic cleavage alterations (Tsen, 2007). In animal and human studies, these compounds appear to compromise cell division by disrupting the spindle-forming apparatus, leading to multipolar spindles, unequal divisions, and chromosome lagging (Kusyk and Hsu, 1976; Starrock and Nunn, 1975). Considering these findings, and considering that, due to our exclusion criteria, only cycles in which more than six follicles were produced were included in this study, we could hypothesize that general anesthesia with sevoflurane has deleterious effects on embryo development.

To our knowledge, this is the first study to evaluate the effects of the combination of fentanyl, propofol and sevoflurane in ICSI outcomes. However, a weakness of this study is that the combined use of propofol to induce general anesthesia, which could create a confounding factor related to propofol’s adverse effects in the analyzed cycles. Propofol is a 2,6 disopropylphenol used as a short-acting intravenous anesthetic agent, suitable for induction and maintenance of anesthesia, and its accumulation in follicular fluid has also been detected (Christiansen et al., 1999; Coetsiers et al., 1992). Christiansen et al. (Christiansen et al., 1999) reported that the diffusion and accumulation of propofol in follicular fluid were time-dependent in relation to the dose of propofol administered. Moreover, Alsalih et al. (Alsalih et al., 1997) reported a significant reduction in the ability of mouse oocytes, exposed to concentrations of 10 μg/mL of propofol for 30 minutes, to mature in vitro. However, no effects were observed in fertilization and cleavage rates compared with the controls. Another study addressed the potentially adverse effects of propofol on the ability of oocytes to fuse with spermatozoa (Talane et al., 1998). The investigators showed that oocytes exposed to concentrations superior to 0.4 μg/mL had a significant decrease in fusion rates. Another study correlated the accumulation of propofol in follicular fluid with negative effects on early embryo development (Coetsiers et al., 1992). However, although propofol concentrations in the follicular fluid are related to the amount administered and to the duration of administration, there is no evidence that propofol can have any significant adverse effect on pregnancy rates. Nevertheless, considering that the use of propofol is often associated with general anesthesia through halogenated agents, it is difficult to isolate one from another and analyze their independent effects. In a further analysis, this study divided ICSI cycles into two groups according to the mean period of anesthesia...
per aspirated follicle, in an attempt to suggest a cut-off point at which general anesthesia can lead to ICSI cycle impairment. Our results found a negative influence of general anesthesia on embryo development and pregnancy rate when the mean period of anesthesia per aspirated follicle was greater than two minutes, suggesting that the timing of the exposure to anesthetic agents plays an important role in ICSI outcome. The period of exposure to anesthesia has been assessed in a study performed by Boyers et al. (Boyers et al., 1987), which compared the in vitro fertilization rates of paired first- and last-recovered human oocytes that were retrieved under general anesthesia. They observed that when the time of retrieval from the first to the last oocyte elapsed up to five minutes, the first- and last-recovered oocytes had different rates of cleavage, whereas for periods greater than five minutes, the difference in fertilization rates between first and last oocytes was significant. However, the investigator found no significant differences in cleavage rates for first- and last-recovered oocytes that fertilized, regardless of the exposure interval. Limited recent information is available about the optimal anesthetic technique for IVP. Many different drugs may be used, and it is difficult to assess whether a specific anesthetic carries more risks than others because many other factors, such as maternal age, semen quality, and endometrial receptivity, may influence the IVP success rate. Our findings suggest that the period of exposure to general anesthesia, with the combination of fentanyl, propofol and sevoflurane, has adverse effects on ICSI outcomes. It could be hypothesized that exposure to this anesthetic combination for periods longer than two minutes per follicle may have deleterious effects on oocyte competence, compromising embryo development and pregnancy potential in ICSI cycles. (Boyers et al., 1987) 

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