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Propofol concentrations in follicular fluid during general anaesthesia for transvaginal oocyte retrieval

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Propofol (Diprivan®) is an i.v. anaesthetic used for general anaesthesia. The purpose of this study was to measure the propofol concentration in arterial blood and follicular fluid in patients during transvaginal oocyte retrieval. After approval by the University Ethics Committee, 30 women participated in this prospective study. Following induction of anaesthesia with 0.5 mg alfentanil and 2 mg.kg^{-1} propofol i.v., a continuous infusion of propofol at 10 mg.kg^{-1}.h^{-1} was used for maintenance of anaesthesia. Follicular fluid and arterial blood samples were aspirated simultaneously at fixed intervals during the surgical procedure and propofol assayed by high pressure liquid chromatography (HPLC). The mean follicular fluid concentration of propofol increased linearly with time from 0.10 ± 0.02 μg.ml^{-1} to 0.57 ± 0.06 μg.ml^{-1} and was strongly related to the cumulative dose of propofol administered. The absorption of propofol was time-dependent. There was no correlation between the concentration of propofol in the follicular fluid and the arterial blood concentration of the drug. In conclusion, a propofol-based anaesthetic technique resulted in significant concentrations of this agent in follicular fluid, related to the dose administered and to the duration of propofol administration.

Key words: anaesthesia/arterial blood/follicular fluid/oocyte/propofol

Introduction

Although propofol (2,6-diisopropylphenol) is frequently used as a general anaesthetic for ultrasound procedures, its use during transvaginal oocyte retrieval is currently being debated. Depypere *et al.* (1991) and Janssenswillen *et al.* (1997) reported a dose- and time-dependent detrimental effect of propofol on the fecundability of mouse oocytes. These findings have been confirmed in two recent papers, where it was also shown that maturation rate was decreased by high propofol concentrations (Alsalili *et al.*, 1997) and cleavage to blastocyst stage was inhibited (Tatone *et al.*, 1998). On the other hand, several authors found no detrimental effects or negative outcome in human or animal in-vitro fertilization (IVF) when propofol was used (Palot *et al.*, 1988; Imoedemhe *et al.*, 1992; Kowalczyk *et al.*, 1993). Sia-Kho *et al.* (1993) even suggested that propofol might be beneficial for the cleavage of oocytes and for the fertilization rate. Borsatti *et al.* (1993) and Rosenblatt *et al.* (1997) reported in retrospective studies no difference in implantation rate or pregnancy rate when propofol was compared with other anaesthetic techniques for oocyte retrieval. Pierce *et al.* (1992) confirmed the lack of difference between the pregnancy rates after propofol or after thiopental following a gamete intra-Fallopian transfer. However, Vincent *et al.* (1995) reported a lower clinical and ongoing pregnancy rate following a propofol/nitrous oxide anaesthesia compared with an isoflurane/nitrous oxide anaesthesia for laparoscopic pronuclear stage transfer.

This study evaluated the propofol concentrations in follicular fluid during general anaesthesia. Coetsier *et al.* (1992) had already demonstrated that propofol accumulation in follicular fluid was time dependent. Their conclusion was based on a pharmacokinetic profile using venous blood samples. However, it has been shown in animal studies that arterial and venous blood sampling may lead to a considerable difference in drug concentrations (Chung *et al.*, 1997; Krejcie *et al.*, 1997) and that arterial sampling may be more accurate for the evaluation of distribution, elimination and pharmacodynamic effects (Chiou, 1989). Since the accumulation of propofol in follicular fluid might have effects on IVF and early embryo development and thus might have important clinical and ethical implications, it was thought interesting to correlate follicular fluid propofol concentrations to arterial blood samples, since this information may further restrict the amount of propofol ultimately administered during anaesthesia. We therefore studied the propofol concentration in arterial blood and follicular fluid in patients undergoing transvaginal oocyte retrieval.

Materials and methods

The study protocol was approved by the University Ethics Committee on Human Research and written informed consent was obtained from all patients. Thirty women, American Society of Anesthesiologists physical status I–II, agreed to participate in this prospective study. After hormonal stimulation, pre-ovulatory oocytes were harvested via ultrasound guided transvaginal oocyte retrieval for conventional IVF or intracytoplasmic sperm injection (ICSI).

The patients received no premedication. An 18-gauge catheter was inserted in one arm and 500 ml Hartmann's solution was used as maintenance infusion. Induction of general anaesthesia (time = T_0) consisted of pre-oxygenation by face mask, followed by 0.5 mg

alfentanil (Rapifen®; Janssen Pharmaceuticals, Beerse, Belgium) and 2 mg.kg^{-1} propofol (Diprivan®; Zeneca, Manchester, UK) i.v. Anaesthesia was maintained with a continuous infusion of propofol at 10 mg.kg^{-1}.h^{-1} i.v. with additional 20 mg increments if necessary. Dosing and times of administration of these propofol supplements were recorded to the nearest minute. Ventilation was controlled by face mask with the patient breathing a 50% O_2–air mixture. Immediately after induction, a 20 gauge arterial catheter was inserted in the radial artery. Follicular fluid and arterial blood samples were aspirated simultaneously at 10, 15, 20, 30 and 40 min after the initial administration of propofol. The follicles whose follicular fluid was used to determine the follicular fluid concentration of propofol were aspirated separately and the aspiration system was flushed between aspirations. The infusion of propofol was discontinued at the beginning of the aspiration of the last follicle.

Blood samples were collected in oxalated tubes and stored as whole blood at 4°C. Follicular fluid was centrifuged at 1800 *g* for 5 min, to eliminate cellular components, e.g. cumulus cells, and frozen at –20°C. Follicular fluid samples that were macroscopically contaminated with blood were discarded. All blood and follicular fluid samples were analysed within 12 weeks of collection.

The concentration of propofol in blood and follicular fluid was determined by high-performance liquid chromatography (HPLC) with separation on a RP-18 column (Merck, Darmstadt, Germany) with acetonitrile (Lab-Scan Ltd, Stillorgan Industrial Park, Dublin, Ireland) (60)–water (40)–orthophosphoric acid (Merck) (0.2) after solid phase extraction on a cyclohexyl column (Varian, Harbor City, CA, USA), with thymol (Merck) as internal standard and fluorescence detection (excitation wavelength: 276 nm, emission wavelength: 310 nm). Further details of the HPLC method are available from the corresponding author. Separation of the propofol from the solvent front and the internal standard was confirmed on chromatograms performed earlier.

Statistical analyses included non-parametric tests (paired Wilcoxon test and Spearman rank correlation) and analysis of variance (ANOVA). Data are expressed as mean and SEM. All tests were performed at a significance level of 0.05.

Results

The mean age of these 30 patients was 33 ± 1 year, their height 163.9 ± 1.4 cm and their weight 70.8 ± 2.8 kg. The average number of oocytes retrieved per oocyte retrieval was 12 ± 1 (range: 3–24). The duration of the procedure ranged from 22 to 45 min. The mean dose of propofol administered per patient was 570 ± 22 mg.

Even with a continuous infusion of 10 mg.kg^{-1}.h^{-1} of propofol, 24 patients (80%) moved when the speculum was placed or the aspiration needle was inserted and needed one or more additional doses of propofol. In seven patients (23%), an additional bolus of 0.5 mg alfentanil (opioid) was necessary.

Six follicular fluid samples were macroscopically contaminated with blood and were discarded. Data from two patients were excluded from analysis because of lost or coagulated blood samples. In all 78, concomitant blood and follicular fluid samples were analysed, as in several patients the surgical procedure lasted <30 min.

The concentration of propofol in arterial blood ranged from 1.40 to 12.10 μg.ml^{-1}. The mean concentrations of propofol in arterial blood are listed in Table I. Concentrations of propofol in follicular fluid were detectable within 10 min of i.v. propofol administration and ranged from 0.04 μg.ml^{-1} to 0.21 μg.ml^{-1}. Except in four patients, the concentration of propofol in follicular fluid steadily increased with the duration of propofol infusion (Table I). The amount of propofol present in follicular fluid was strongly related to the cumulative dose of propofol administered (Figure 1). The concentration of propofol in the follicular fluid did not correlate with the arterial blood concentration of the drug (Figure 2) nor to the plateau

Table I. Propofol concentration in blood and follicular fluid during transvaginal oocyte retrieval (mean ± SEM)

Time after induction (min)	Propofol concentration blood (μg.ml^{-1})	Propofol concentration follicular fluid (μg.ml^{-1})
10	4.89 (± 0.58)	0.10 (± 0.02)
15	6.32 (± 1.01)	0.17 (± 0.04)
20	5.17 (± 0.45)	0.34 (± 0.05)
30	5.99 (± 0.46)	0.43 (± 0.05)
40	4.10 (± 0.27)	0.57 (± 0.06)

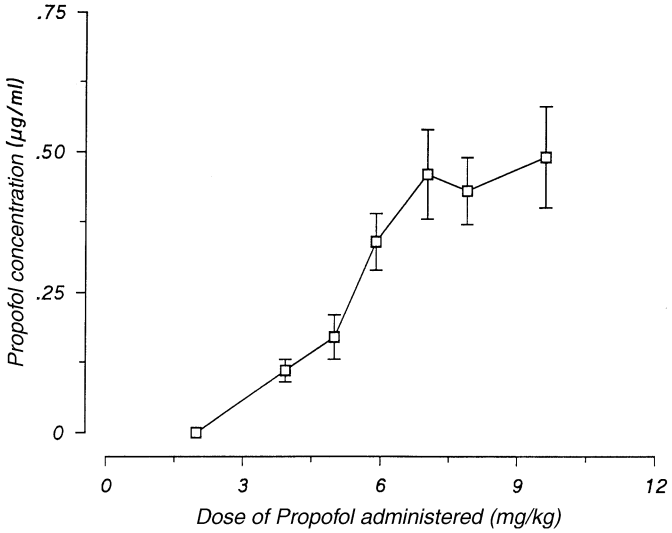

Figure 1. Relation between follicular fluid propofol concentration (μg.ml^{-1}) and weight-normalized cumulative dose of propofol administered (mg.kg^{-1}).

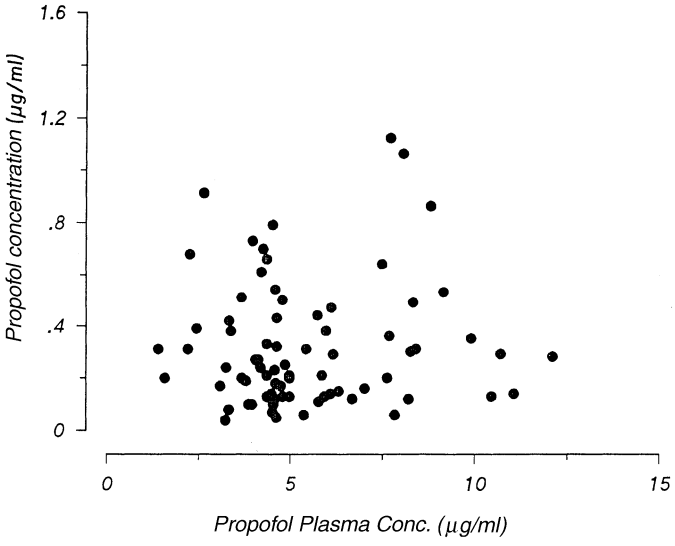

Figure 2. Scattergram of arterial and follicular fluid concentrations of propofol (μg.ml^{-1}).

concentration of propofol achieved during the continuous infusion of this agent. The absorption of propofol in follicular fluid was time-dependent ($P < 0.001$) with an apparent clearance ratio from the blood to the follicular fluid of $6.04 \pm 0.01\%$ (range 1.9–15.5) as estimated from areas under the drug concentration–time curves in blood and follicular fluid.

Discussion

The main findings in this study were the time-dependent diffusion and accumulation of propofol in follicular fluid in relation to the dose of propofol administered. In an attempt to elucidate the relationship between drug concentration in blood and that in follicular fluid following a constant-rate infusion, no correlation between either the individual arterial or steady-state blood concentrations of propofol and the related concentration of the drug in follicular fluid was found. This suggests that the follicular fluid should be assigned to the deep peripheral compartment of the three-compartment open model for propofol. The process of propofol transfer from blood to follicular fluid was very slow with an apparent clearance ratio of 6%. As only two to four samples were available from each patient, the present data did not allow an estimation of the pharmacokinetic models for simulating drug concentrations in follicular fluid. These data support Coetsier *et al.*'s observations (1992) of propofol accumulation in follicular fluid, but in contrast to their study, higher blood and follicular fluid concentrations of propofol were found in this study. One possible cause is the administration of a small dose of alfentanil to the patients in this study. Alfentanil has been shown to elevate propofol concentrations (Vuyk 1997), although it is doubtful that such a small dose should have been important. Another cause may be differences in blood sampling. Coetsier *et al.* (1992) sampled venous blood, while in our study arterial blood was taken. Arterial animal blood samples steadily show higher drug concentrations (Chung *et al.*, 1997; Tuk *et al.*, 1998) than venous samples when taken simultaneously. Sampled venous blood is also known to be unreliable for assessing the distribution kinetics of lipophilic anaesthetic agents. Other factors such as haemodynamic status, depth of anaesthesia or patient recruitment may be responsible for the differences found in follicular propofol concentration reported in the two studies.

The mean follicular fluid propofol concentration observed in this study was 0.43 $\mu g.ml^{-1}$ after 30 min and 0.57 $\mu g.ml^{-1}$ after 40 min. Propofol was shown to be a parthenogenetic activator for denuded mice oocytes at a concentration of 0.4 $\mu g.ml^{-1}$ after 30 min incubation (Janssenswillen *et al.* 1997). Thus, the concentration of propofol in follicular fluid found here might induce deleterious effects on subsequent cleavage and fertilization.

Despite controversial reports with regard to the influence of propofol anaesthesia on implantation rates and clinical pregnancy rates in humans (Pierce *et al.*, 1992; Vincent *et al.*, 1995; Rosenblatt *et al.*, 1997), caution seems warranted with the use of propofol for oocyte retrieval. The potential deleterious effects of this agent could be minimized by modifying the anaesthetic technique in order to limit the dose of propofol administered to <4 $mg.kg^{-1}$ (Figure 1).

In conclusion, a propofol-based anaesthetic technique resulted in significant concentrations of this agent in follicular fluid, related to the dose administered and to the duration of propofol administration. The concentrations of propofol in follicular fluid were larger than those shown to exert parthenogenetic activity in mice oocytes. In the light of the deleterious effects of propofol on oocyte fertilization decribed in mouse models by others, the retrieved oocytes should be washed free of propofol. Taking into account the potential negative effects on human artificial reproductive technology, the total dose of propofol administered during anaesthesia should be strictly limited.

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