

The effect of propofol anaesthesia on oocyte fertilization and early embryo quality

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Propofol, frequently used for i.v. induction of anaesthesia in assisted reproduction procedures, has been suspected of damaging oocytes. Concentrations of propofol have recently been shown to increase in follicular fluid during oocyte retrieval. Our study was designed to assess whether exposure to increasing concentrations of propofol has a measurable effect on in-vitro fertilization, cleavage and embryo development. A cohort of 130 women underwent i.v. anaesthesia using propofol and fentanyl. Time of anaesthesia from i.v. injection of propofol was measured, as were the doses of the two drugs. In 32 women expected to have more than 15 oocytes retrieved, first, middle and last oocytes were cultured separately. The mean time from i.v. injection to first follicle aspiration was 200 s. The mean time for the aspiration of each additional oocyte was 17.6 s. In 10 out of 11 cases where follicular fluid concentrations of propofol were measured, there was an increase from the first to the last follicle, but no difference was found in the ratio of mature to immature oocytes. Nor were any differences found in fertilization, cleavage and embryo cell number. In so far as in-vitro development reflects embryo quality, we conclude that the time elapsed between retrieval of the first and last oocyte does not affect oocyte quality.

Key words: embryo quality/oocyte fertilization/propofol

Introduction

General anaesthesia with i.v. agents seems to be the most popular form of pain control for transvaginal oocyte retrieval in assisted reproduction (van der Ven *et al.*, 1988; Ben-Shlomo *et al.*, 1992). Concern has been expressed as to possible detrimental effects of anaesthetic and analgesic agents on the results of assisted reproduction treatments (Batzner *et al.*, 1994). Indeed, the concentrations of some agents have been measured in the aspirated follicular fluid (FF; Palot *et al.*, 1988; Wikland *et al.*, 1990; Coetsier *et al.*, 1992; Soussis *et al.*, 1995), the latest of which was propofol (Christiaens *et al.*, 1999). Direct

observations in animal models also tested the possible toxic effect on oocyte fertilization and early embryonic development (Alsaili *et al.*, 1997; Janssenswillen *et al.*, 1997).

Propofol is chemically unrelated to earlier anaesthetic drugs. This highly lipophilic agent has a fast onset and short, predictable duration of action due to its rapid penetration of the blood–brain barrier and distribution to the central nervous system (CNS; Kanto and Gepts, 1989). It is very popular for a host of ambulatory procedures including oocyte retrieval (Rosen *et al.*, 1991; Coetsier *et al.*, 1992; Moscona *et al.*, 1995; Tontisirin *et al.*, 1996). Although some reports have not found any detrimental effect of propofol on assisted reproduction outcome, a recent report challenged its safety (Tatone *et al.*, 1998). Recently it has been shown that when total i.v. anaesthesia is maintained with continuous propofol pump, a gradual, time dependent, linear increase of its concentrations is observed in FF (Christiaens *et al.*, 1999). Interestingly, no possible biological effects were detected. In an attempt to document possible differences in fertilization rates and early embryo development potentially attributable to propofol, we separated first aspirated from last aspirated oocytes during propofol/fentanyl induced anaesthesia for oocyte retrieval.

Materials and methods

The study group included 130 unpremedicated women scheduled for oocyte retrieval. Ovarian stimulation was achieved as previously described (Shalev *et al.*, 1995; Ben-Shlomo *et al.*, 1997). Since we did not intervene by any non-standard or new method, no Institutional Review Board approval was sought. The general anaesthesia protocol consisted of i.v. fentanyl 0.5–1.0 mg followed after 2 min by i.v. propofol 1.5–2.0 mg/kg. Monitoring was maintained as previously reported (Ben-Shlomo *et al.*, 1999). An adequate level of anaesthesia was maintained by repeated boluses of propofol as clinically appropriate. Oocyte retrieval was performed transvaginally as previously described (Shalev *et al.*, 1995). Embryo transfer was carried out 48–72 h after retrieval.

The time elapsed from the initial administration of propofol to the aspiration of the last follicle was recorded. Vaginal aseptic cleansing was done under anaesthesia. In 32 patients predicted to have at least 15 oocytes, the oocytes were divided and kept in groups according to the sequence of aspiration. Thus, about a third of the oocytes were designated 'early', a third were designated 'last' and all the rest were called 'intermediate'. The three respective groups were handled and kept separately until embryo transfer (or cryopreservation). In 17 patients out of this group FF from the first and last follicles were collected separately. Special care was taken to ascertain that the last aspirated follicle would be approximately the same size as the first. If not blood tainted (11 cases), FF were kept frozen (–20°C) in glass vacuum tubes, covered with aluminium foil for later analysis of

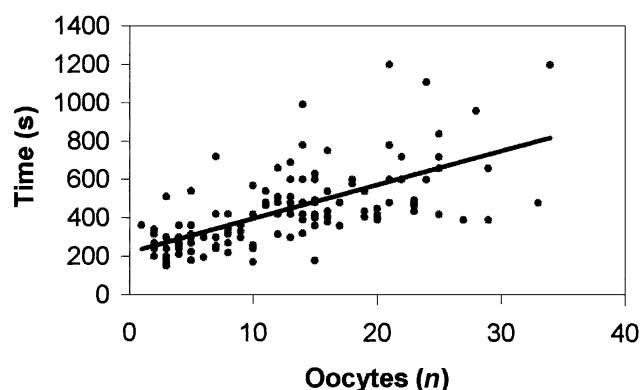


Figure 1. The time elapsed from the injection of propofol to the aspiration of the last follicle in 130 cycles of oocyte retrieval for in-vitro fertilization, plotted against the number of oocytes retrieved.

propofol concentrations. Propofol concentrations were determined using modified high-pressure liquid chromatography (Knibbe *et al.*, 1998). In brief, for the mobile phase, a mixture of acetonitrile/distilled water/trifluoroacetic acid (70:30:0.1 v/v/v) was used. Calibrations with varying dilutions of propofol in either FF or distilled water were done using 1% propofol vials for i.v. injection. No difference was found between the two solvents. Aliquots of 0.5 ml from each sample were mixed vigorously with 1 ml acetonitrile and centrifuged. A sample of 60 μ l from the supernatant was passed through Prodigy 5 μ m ODS (3), 250 \times 4.6 mm column (Phenomenex Inc., Torrance, CA, USA). Retention time was 6.38 min. The limit of detection was 3 ng/ml and coefficient of variation at 125 ng/ml was 7%.

Power considerations indicated that if the effect of propofol was expected to decrease fertilization rate after intracytoplasmic sperm injection (ICSI) from 75 to 60%, then a cohort of 151 oocytes would be needed in each group for a reliable negative conclusion (power of 0.8; $P < 0.05$). Statistical calculations included regression analysis, Student's *t*-test (paired), Mann-Whitney *U* test and χ^2 test as applicable.

Results

The duration of oocyte retrieval procedures, from administration of propofol to conclusion of aspiration against the respective number of oocytes retrieved, is presented in Figure 1. The longest procedure lasted 20 min and the shortest 3.5 min. The mean time to the aspiration of the first oocyte was 200 s and the calculated mean rate of oocyte retrieval was one in 17.6 s. The slope of the curve was highly significant ($R^2 = 0.4$; $P = 2.5 \times 10^{-6}$).

Table I presents the in-vitro results in the three groups of oocytes according to the time of their retrieval. There was a significant difference in the proportion of immature oocytes, probably reflecting the tendency to aspirate more small follicles towards the end of the procedure. However, there were no differences in the rates of fertilization and cleavage between the first and last aspirated oocytes. The quality of the embryos, as reflected in the mean cell number at embryo transfer (or freezing), was also comparable between the groups. It was known from previous work that the number of blastomeres accurately reflected the overall embryo quality.

Figure 2 shows the changes in propofol concentration according to total time of anaesthesia from the first to the last

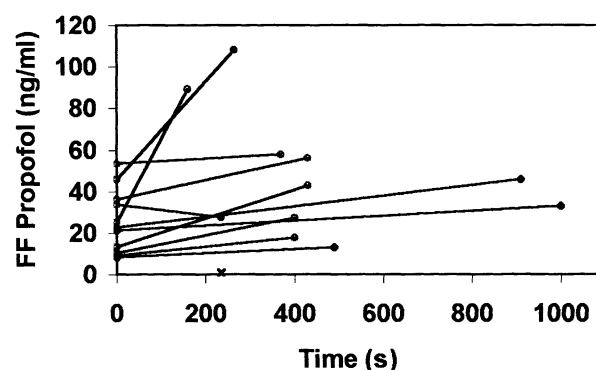


Figure 2. Change in follicular fluid (FF) propofol concentrations in individual patients from the first aspirated follicle to the last aspirated one in 11 women undergoing oocyte retrieval for in-vitro fertilization.

Table I. In-vitro results of intracytoplasmic sperm injection in three groups of oocytes, divided by the sequence of retrieval (oocytes were retrieved from 32 patients, each predicted to have ≥ 15 oocytes)

	First	Middle	Last	<i>P</i> value
Oocytes (<i>n</i>)	199	187	204	—
Immature oocytes (%)	36 (18.0)	40 (21.4)	58 (28.4)	0.04
M2 oocytes (injected) (<i>n</i>)	163	147	146	—
Fertilized (%)	125 (76.7)	105 (71.4)	117 (80.1)	NS
Cleaved (%)	110 (67.5)	98 (66.7)	101 (69.2)	NS
Cells/embryo (SD)	4.6 (1.6)	4.5 (1.6)	4.6 (1.8)	NS

NS = not significant.

aspirated follicular fluid in 11 women. Except for one, all had a higher concentration of propofol in the last FF as compared to the first ($P < 0.01$). However, there was no correlation between the absolute concentrations or the percentage of increase and the dose given to the patients or the time elapsed between the first and the last follicular aspirations.

Discussion

In this prospective study we examined the possible effect of exposure to the i.v. anaesthetic drug propofol on the results of in-vitro fertilized oocytes. In an earlier study, performed during the era of laparoscopic oocyte retrieval, Hayes *et al.* (1987) used comparable methodology to examine the effect of CO₂ pneumoperitoneum and anaesthesia duration on the same parameters. They concluded that both might have adversely affected fertilization. A later study, which compared the vaginal route of retrieval with the laparoscopic one (Lavy *et al.*, 1988) concluded that anaesthesia may add a further detrimental effect beyond that of CO₂ pneumoperitoneum.

The shift in popularity of oocyte retrieval technique to the exclusive use of the transvaginal route has significantly shortened the duration of the procedure as well as the degree of pain involved. Nevertheless, concerns still exist regarding possible detrimental effects of anaesthetic agents on the quality of oocytes and the corresponding embryos. In this regard, there are also some studies in animals which give some support to this concern (Janssenswillen *et al.*, 1997). A recent study (Christiaens *et al.*, 1999) gave a solid demonstration of the

fact that follicle-contained oocytes are exposed to increasing concentrations of propofol as anaesthesia continues. Although we observed the same trend, we could not demonstrate a correlation between the concentrations in FF and the duration of anaesthesia. This may be attributed to the relatively small number of patients tested for FF concentrations of propofol ($n = 11$) or the discontinuous mode of propofol administration that we employed. In this regard it is striking that the total dose of propofol which we administered to our patients was lower than that reported by Christiaens *et al.* (1999), as was the mean operation time. Whereas they reported doses of propofol up to 10 mg/kg, we never had a dose higher than 5 mg/kg, the mean being $2.54 (\pm 0.76)$ mg/kg. It is not surprising therefore, that the concentrations of propofol that we measured in FF were markedly lower. Nevertheless, motivated by the concern that exposure of the last retrieved oocytes to more propofol compared to early retrieved oocytes could damage the quality of the resulting embryos, we recorded their respective in-vitro outcome. As far as such a limited observation as ours can predict lasting only during the extracorporeal time of incubation, this is not the case. A caveat, however, is the difference in the overall duration and cumulative doses of propofol given by us compared with those reported by Christiaens *et al.* (1999).

In conclusion, we did not demonstrate a detrimental effect of the rising concentrations of FF propofol on oocyte quality. However, we cannot rule out the possibility that the short duration of our retrieval procedures and the consequent low FF propofol concentrations contributed to this result.

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