The effect of the anaesthetic, Propofol, on in-vitro oocyte maturation, fertilization and cleavage in mice

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Propofol is a common anaesthetic agent used for oocyte retrieval procedures during in-vitro fertilization (IVF). The effect of Propofol in vitro on mouse oocyte maturation, fertilization and embryo cleavage was studied. In this study, 551 cumulus-free and 222 cumulus-enclosed oocytes from mice stimulated with pregnant mare's serum gonadotrophin (PMSG) were incubated for 30 min in medium containing 0, 100, 1000 or 10 000 ng/ml of Propofol prior to in-vitro maturation. Also, 325 cumulus-enclosed oocytes from mice stimulated to ovulate with PMSG/human chorionic gonadotrophin (HCG) were incubated for 30 min in similar concentrations of Propofol prior to IVF. Maturation, fertilization and cleavage rates were compared. A significant decrease in the in-vitro maturation rate was observed only when the cumulus-free and cumulus-enclosed oocytes were exposed to 10 000 ng/ml Propofol (P < 0.0074 and P < 0.0001 respectively). Fertilization and embryo cleavage rates were not significantly different compared with the controls. These findings give some reassurance with respect to human IVF. However, further studies on the potential effects of Propofol on implantation and pregnancy outcome following IVF are needed.

Key words: anaesthesia/cleavage/in-vitro fertilization/oocyte maturation

Introduction

Ultrasound-guided transvaginal oocyte retrieval (TVOR) has become widespread as it simplifies in-vitro fertilization (IVF) treatment, enabling most patients to be treated on a same day, outpatient basis (Dellenbach et al., 1985; Wikland et al., 1985). The procedure is carried out under either general, epidural or local anaesthesia (Raftery and Sherry, 1992; Botta et al., 1995). Propofol (diprivan) is one of the commonest drugs used for anaesthesia in this situation.

Propofol is a 2,6-diisopropylphenol which is a short-acting i.v. anaesthetic agent suitable for induction and maintenance of anaesthesia. It has a rapid onset of action of ~30 s following an i.v. injection. The recovery from anaesthesia is also rapid. The mechanism of action of Propofol, like all general anaesthetics, is poorly understood. It has been reported that Propofol inhibits polysynaptic excitatory neurotransmission in the olfactory cortex and spinal cord which is thought to be mediated by the N-methyl-D-aspartate receptor channel, in addition to the potentiation of γ-aminobutyric acid-mediated synaptic inhibition in a variety of systems (Yamakura et al., 1995). Propofol was shown to reduce post-operative nausea and vomiting compared with inhalational anaesthesia; requirements for antiemetic therapy and unplanned admission for overnight stay in hospital were also reduced (Raftery and Sherry, 1992).

Propofol has been reported to accumulate in the follicular fluid (FF) (Palot et al., 1988). Propofol concentrations in FF seem to increase steadily throughout the TVOR procedure (Coetsier et al., 1992). It has been suggested that Propofol may have deleterious effects on reproductive outcome following IVF. It was suggested that there is a dose- and time-dependent toxic effect of Propofol on the fertilization of mouse oocytes (Depypere et al., 1991). Recently, Janssenswillen et al. (1996) assessed the effect of Propofol on fertilization and embryo development using a mouse IVF model. They showed a significant decrease in blastocyst formation when Propofol was added to the medium. They concluded that Propofol is deleterious for subsequent cleavage and that the exposure of the unfertilized oocytes to Propofol results in a high incidence of parthenogenetic activation.

In this study, we have sought to expand on this work and assess in more detail the effects of the anaesthetic Propofol on oocyte and embryo development. A controlled comparative study was conducted to examine the effect of Propofol, using different concentrations, on the in-vitro maturation of cumulus-free and cumulus-enclosed oocytes. The rationale for this approach was to ascertain whether Propofol has a direct or indirect effect, as it is known that cumulus cells play a part in the control of oocyte maturation through communication via gap-junctions. In addition the effect of different concentrations of Propofol on the IVF and cleavage of cumulus-enclosed mouse oocytes was investigated, as an appropriate model for human IVF.

Materials and methods

In-vitro oocyte maturation

Female B6C3 mice (aged 3 weeks) were injected by the i.p. route with 5 IU pregnant mare's serum gonadotrophin (PMSG) (Intervet, Cambridge, UK) 48 h prior to in-vitro oocyte maturation experiments. On the day of the experiment, the mice were killed by cervical dislocation and dissected.

Using sterile technique, the ovaries were dissected and transferred to a Petri dish with M2 medium (Sigma, Poole, UK) containing one of the following concentrations of Propofol (Zeneca, Macclesfield, UK): 0, 100, 1000 and 10 000 ng/ml. The follicles were punctured using a 25-gauge needle. Cumulus-oocyte complexes were collected.
and transferred to drops of M2 with the respective Propofol concentrations. Preantral follicles were avoided. Cumulus cells were either removed using a pulled pipette for the cumulus-free oocyte experiments or left intact for the cumulus-enclosed oocyte experiments. Oocytes were isolated and incubated for 30 min in one of the four M2 culture media containing different concentrations of Propofol at 37°C. After incubation for 30 min, oocytes were washed through 3–4 drops of pre-equilibrated minimal essential medium (MEM; Sigma) supplemented with 5% (v/v) fetal bovine serum (Sigma) and 0.25% (w/v) pyruvic acid (Sigma) and incubated at 37°C in 5% CO₂. After incubation for 3 h, the oocytes were assessed for germinai vesicle breakdown (GVB) and progression to metaphase I (MI). In the case of cumulus-enclosed oocytes, the cumulus cells were dissected off using a pulled pipette. Oocytes were re-incubated overnight. The stage of meiotic maturation after 18 h of incubation was assessed for progression to metaphase II (MII).

**In-vitro fertilization**

Female B6C3 mice (3–6 weeks) were injected by the i.p. route with 5 IU PMSG (Intervet) and human chorionic gonadotrophin (HCG; Intervet) 48 h apart. Female mice were killed 18–20 h post-HCG, by cervical dislocation, and dissected. Oviducts were isolated and transferred to one of the M2 media containing different concentrations of Propofol, as described above. Cumulus masses were released from the oviduct using a sharp needle. The cumulus masses were incubated in the medium for 30 min at 37°C and then transferred to drops of M16 medium (Sigma) under oil. In M16, they were washed in three drops and then incubated at 37°C in 5% CO₂, awaiting insemination.

A male B6C3 mouse was killed, by cervical dislocation, and dissected. The caudal epididymis and vas deferens were transferred to a drop of M16 under oil. Spermatozoa were expressed and dispersed, then incubated at 37°C in 5% CO₂ for 1 h for capacitation. The sperm sample was then diluted to achieve a final concentration of 2×10⁶/ml. Insemination was performed by taking out previously incubated drops of M16 under oil and replacing them with drops of the sperm concentration (2×10⁶/ml). After 4 h incubation, cumulus cells were dissected off the oocytes using a pulled pipette. The oocytes were transferred to a fresh drop of M16 and observed for fertilization and cleavage.

**Results**

**In-vitro oocyte maturation**

A total of 773 oocytes were incubated in the culture media. Of these, 702 (90.8%) oocytes underwent GVB after 3 h and progressed to MI. However, only 473 (61.2%) oocytes progressed to MII in 18 h. Cumulus-enclosed oocytes achieved a significantly higher maturation rate compared with cumulus-free oocytes (73.4 versus 56.3%; \( P < 0.0001 \)) (Tables I and II).

In-vitro oocyte maturation in culture media containing Propofol resulted in a significant reduction in the MII formation rate only at a concentration of 10 000 ng/ml (Tables I and II).

**Cumulus-free oocytes**

In these experiments, 551 cumulus-free oocytes were incubated for 30 min in the different concentrations of Propofol. Of these, 310 (56.3%) oocytes reached MII (Table I). The reduction in MII formation was only significant \( (P < 0.0074) \) at the 10 000 ng/ml concentration of Propofol (Table I).

**Cumulus-enclosed oocytes**

In these experiments, 222 cumulus-enclosed oocytes were incubated for 30 min in the different concentrations of Propofol. Of these, 163 (73.4%) oocytes reached MII (Table II). The reduction in MII formation was only statistically significant \( (P < 0.0001) \) at the 10 000 ng/ml Propofol concentration (Table II), and was more profound than that in cumulus-free oocytes \( (P < 0.0001 \text{ versus } P < 0.0074) \).

**In-vitro fertilization**

A total of 325 cumulus-enclosed oocytes were used for these experiments. Following 30 min incubation of the oocytes in the 0, 100, 1000 and 10 000 ng/ml Propofol concentrations in M2 media, they were transferred to M16 media where they were washed and inseminated. There was no significant difference between the fertilization and cleavage rates at the different Propofol concentrations (Table III).

**Discussion**

The accumulated effect of anaesthetic agents on oocyte activation, fertilization, cleavage and quality of embryos has been the subject of several animal and human studies. However,
the success of IVF is influenced by many factors including the aetiology of infertility, female age (Tan et al., 1992), basal follicle stimulating hormone concentration (Toner et al., 1991), ovarian stimulation protocol (Hughes et al., 1992), number of oocytes retrieved, culture conditions, embryos transferred (Sharma et al., 1988) and luteal support (Akande et al., 1996).

In this prospective study, we investigated the effect of the anaesthetic Propofol on oocyte and embryo development *in vitro* in mice. An incubation period of 30 min was chosen in these experiments as this is around the maximal time that oocytes might be exposed to anaesthetic agents during human oocyte retrieval, although a longer duration of exposure may be relevant to human IVF under certain circumstances, such as transport IVF. Spontaneous in-vitro oocyte maturation was adversely affected only when the oocytes were incubated in medium containing 10,000 ng/ml of Propofol. This effect was seen in both cumulus-free and cumulus-enclosed oocytes and was more pronounced in the cumulus-enclosed oocytes. No effects were seen at lower concentrations of Propofol.

These findings would suggest that oocytes exposed to a high concentration of Propofol may not mature adequately *in vitro*. However, the Propofol concentration at which this effect was seen in the mouse is much higher (maybe 20–30 times; M.Alsalili, S.Fleming, S.Thornton et al., unpublished observations) than the FF Propofol concentrations that have been reported in human IVF (Palot et al., 1988; Coetsier et al., 1992). The accuracy of the mouse embryo assay with regard to its ability to predict human reproductive toxicity has been challenged because of its low sensitivity however (Fleming et al., 1987; Davidson et al., 1988).

Findings of impaired maturation may help to explain some cases of human infertility. Recently, it was shown that ~20% of apparently unfertilized oocytes are penetrated by spermatozoa but have arrested at an early stage of the fertilization process (Van Blerkom et al., 1994). Arrest at this stage may be due in part to paternal factors such as a lack of oscillin (Parrington et al., 1996), or abnormalities in the paternal centrosome (Simerly et al., 1995b), but it may also be due to abnormalities in oocyte maturation resulting in inability to trigger calcium oscillations (Carroll et al., 1996). Cumulus-enclosed oocytes were found to undergo in-vitro maturation at a significantly higher rate compared with cumulus-free oocytes. Previous studies are consistent with these findings (Downs et al., 1988; Bilodeau et al., 1993). These findings suggest that cumulus cells produce a positive factor in response to ligand treatment, that bypasses their negative influence, to bring about meiotic maturation. Oocyte development may be partly dependent on the cumulus cells via their gap junctions, paracrine communication and interactions with elements of the extracellular matrix (Eppig, 1992). The positive contribution of the cumulus cells to oocyte development has been utilized in human IVF, where cumulus-oocyte complexes and embryos are grouped together to improve embryo quality and pregnancy rate (Moessner and Dodson, 1995; Almagor et al., 1996).

Similarly, the use of cumulus cells as a co-culture system seems to improve the quality of embryos and enhance their development, and may improve pregnancy rates following IVF (Mansour et al., 1994; Freeman et al., 1995).

Fertilization and cleavage rates of mouse oocytes were not affected with the different concentrations of Propofol in this study. This is in contrast to a previous study by Depypere *et al.* (1991) where they investigated the influence of Propofol on IVF in mice. They suggested that there is a dose- and time-dependent toxic effect of Propofol on the fertilization of mouse oocytes. Unfortunately, this study was in abstract form with very little detail of the methodology used, making it difficult to compare with our study. However, they used a different strain of mice which may account for the difference in response. Consistent with our findings, they showed no detrimental effect of Propofol on subsequent cleavage and blastocyst formation. In a similar study design, Kowalczyk *et al.* (1993) measured FF Propofol values in 19 patients undergoing gamete intra-fallopian transfer. They also incubated 2-cell mouse embryos in various concentrations of Propofol and found that even five times the peak FF concentration did not interfere with blastocyst development when compared with controls. In contrast, Janssenswillen *et al.* (1996) reported a significant decrease in blastocyst formation when oocytes were incubated for 30 min in medium containing 50, 250, 500, 1000 and 5000 ng/ml of Propofol. They suggested that a brief exposure of cumulus-enclosed oocytes to low concentrations of Propofol was deleterious for subsequent cleavage. Our findings, and those of others (Depypere *et al.*, 1991; Kowalczyk *et al.*, 1993) do not support their conclusion.

In conclusion, our findings suggest that high concentrations of Propofol may be detrimental to *in-vitro* oocyte maturation in mice. This effect however, was only observed at levels which are likely to be supra-pharmacological in human IVF. Propofol did not affect fertilization or cleavage rates. Given the multiple factors which may affect the outcome of IVF we can take some reassurance from these findings. However, caution must be exercised as simple extrapolation from animal data to human applications may prove to be inappropriate as there are species differences in mechanisms and responses to different stimuli (Simerly *et al.*, 1995a). In this study, potential effects on implantation and pregnancy outcome have not been explored and may warrant further studies. Other anaesthetic agents also need to be investigated and may be compared with Propofol.

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**References**


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