Ultrasound covers and sonographic gels are embryo-toxic and could be replaced by non-toxic polyethylene bags and paraffin oil

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The objective of this study was to test the hypothesis that ultrasound covers and sonographic gels, used during vaginal ultrasound, are toxic for mouse embryonic development in vitro. A prospective randomized design was used on pronucleate ova of F1 hybrid CBA×C57Bl female mice. The mice were superovulated with pregnant mare’s serum gonadotrophin and human chorionic gonadotrophin and mated with CBA×C57Bl males. The pronucleate ova were randomly divided between culture media with the addition of commercially available ultrasound covers and sonographic gels in different concentrations. As controls and potential alternatives, plastic polyethylene bags and paraffin oil were tested simultaneously. Embryo-toxicity was assessed by documenting cleavage capacity, blastocyst formation and embryo degeneration in vitro. Exposure of culture medium to the ultrasound covers and sonographic gels tested resulted in a severely reduced cleavage capacity, a high incidence of embryo degeneration and absent or impaired blastocyst formation. This toxic effect could be reduced by high dilutions in vitro. In contrast, plastic polyethylene bags and paraffin oil had no influence on in-vitro development of mouse ova. We conclude that commercially available ultrasound latex covers and sonographic gels are toxic for mouse embryos and can potentially influence embryonic development during infertility treatment. It is safer to perform vaginal ultrasonic measurements using non-toxic paraffin oil (as contact fluid) and plastic polyethylene bags (as ultrasonic cover).

Key words: condoms/gels/mouse embryonic development/embryotoxicity/ultrasound

Introduction
Vaginal ultrasonography is usually performed during ovarian follicular follow-up in infertility treatment and during oocyte retrieval in in-vitro fertilization (IVF). During these ultrasonic examinations, the vaginal probe is protected by an ultrasound cover (or a condom) for hygienic reasons and moistened with gel to improve the quality of the images. Since the sonographic gels and ultrasound covers are in intimate contact with posterior fornix, cervix and cervical mucus, it is important to investigate the possible toxic effects of these gels and covers on mouse embryonic development in vitro.

Only a few studies are available concerning potential toxicity of ultrasonic gels. A decreased sperm motility due to a loss of sperm viability has been reported within the first hour following sperm exposure to 10% sonographic gel (Shimonovitz et al., 1994). Cell lysis due to ultrasonic gel contamination of breast tissue (Molyneux and Coghill, 1994) and a case report of allergic contact dermatitis (Gebhart et al., 1993) have also been reported.

To the best of our knowledge, no information is available on the potentially toxic effects of ultrasonic condoms and gels used in reproductive medicine. However, there is an extensive literature (Hidvegi et al., 1978; Karciglu et al., 1988; Kang et al., 1992) about the pathological effects of talc and starch dusting powders used on latex rubber condoms and surgical gloves. Intrapertioneally, talc and starch can cause serious peritonitis with white miliary nodules, ascites, granulomas and dense adhesions (Huertas et al., 1982; Gutteridge, 1993).

It is possible that ultrasonic gels and talc on covers and condoms pass through the cervix and accumulate on the endometrium, the Fallopian tubes and the ovaries, compromising transport, development and/or implantation of embryos. Therefore, we tested the hypothesis that commercially available ultrasound gels and covers may impair mouse embryonic cleavage and development in vitro, in a randomized controlled study.

Materials and methods
Four to five week old F1 CBA×C57Bl female mice were superovulated with i.p. injections of 5 IU pregnant mare’s serum gonadotrophin (PMSG; Folligon®; Intervet, Brussels, Belgium) and 5 IU human chorionic gonadotrophin (HCG; Pregnyl®; Organon, Oss, The Netherlands) at 1700 h, 48 h apart, mated and checked for copulation plugs the next morning. Mated females were killed by cervical dislocation and their oviducts flushed with HEPES buffered Earle’s medium. The morphologically normal pronucleate ova were pooled (n = 870) and randomly divided over two simultaneous experiments and one control group. The in-vitro culture was performed in human tubal fluid medium (HTF) (Quinn et al., 1985) supplemented with 0.5% bovine serum albumin (BSA).

In the first experiment, 2 ml of culture medium was exposed to 1 cm² of two commercially available ultrasound latex covers: A (Ultra-Cover®; International Medical Products, Zutphen, the Netherlands, treated with Keoflo 7136P-USP absorbable dusting powder) and B (Save-Scan®; Amedic, Sollentunan, Sweden) and 1 cm² of a plastic polyethylene bag (Jemaco, Herent, Belgium). The cover- and bag-fragments were added to the culture medium either for 1, 2 or 4 h prior to addition of the pronucleate ova or continuously during embryo culture (96 h). Medium that had been incubated for 1 h with these fragments was further diluted (1/10, 1/100 and 1/1000), tested for toxicity and compared with untreated control medium.
Toxicity of ultrasound covers and sonographic gels

Figure 1. In-vitro toxicity test of pronucleate mouse ova cultured for 96 h in media exposed to two commercially available ultrasound covers A and B or to a plastic polyethylene bag, for different incubation times. cnt: continuous exposure. Untreated human tubal fluid (HTF) medium was used as control (c).

In the second simultaneous experiment, two sonographic carboxymethylene gels, C (home-made carbopol-gel) and D (Save-Scan®; Amedic) were tested in four dilutions (1/10, 1/100, 1/1000 and 1/10 000 v/v with HTF medium) and were compared with medium under a layer of paraffin oil (Sterop, Paraffinum Liquidum, N.V. PHN-Apr. L. Eykerman, Brussels, Belgium), with medium washed for 1 h with paraffin oil and with untreated control medium.

Both experiments were performed in triplicate and each condition contained 30 pronucleate ova. Cleavage capacity, blastocyst formation and embryonic degenerations were registered after 24 and 96 h of in-vitro culture.

Statistical analysis of differences was performed with χ² test and regression analysis.

Results

Experiment 1 showed that the exposure of culture medium for 1, 2, 4 or 96 h to ultrasound cover A resulted after 24 h of culture in a severely reduced embryo cleavage capacity, i.e. 63, 60, 33 and 0% respectively (P < 0.0001; Figure 1A) and in a high incidence of embryo degeneration, i.e. 33, 23, 63 and 100% respectively (P < 0.0001; Figure 1B). Blastocysts were completely absent after 96 h of culture, even after an exposure time of only 1 h prior to the addition of the pronucleate ova (P < 0.00001 compared to the controls; Figure 1C). After 96 h of culture, degeneration was observed in all embryos cultured in medium exposed to cover B, regardless of previous exposure time (P < 0.0001; Figure 1D).

The severe toxicity of both covers could be reduced and resulted in a normal cleavage rate and blastocyst formation after a 1/1000 dilution for cover A, i.e. 100 and 96% respectively and after a 1/10 dilution for cover B, i.e. 100 and 87% respectively (Figure 2).

In contrast, media containing plastic polyethylene bag fragments, even when these were added continuously to the culture medium (Figure 1), had no influence on embryo cleavage capacity (100%) nor on further development to blastocysts (80%) when compared to control medium, i.e. 93 and 82% respectively (NS).

Experiment 2 showed that media containing 10% of sonographic gels C or D were highly toxic as shown by a 100% embryo degeneration for gel C after 24 h (P < 0.0001; Figure 3B) and no blastocyst formation (0%) for gel D (P < 0.0001; Figure 3C). This toxicity could be reduced, but high dilutions (1/1000) were required for gel C and gel D to restore normal embryo cleavage (100 and 100% respectively; Figure 3A) and to restore normal blastocyst formation (75 and 83% respectively; Figure 3C).

Medium under a layer of paraffin oil or washed for 1 h with paraffin oil had no influence on embryo cleavage (96 and 100% respectively) or development to blastocysts (80% and 87% respectively; Figure 3A,B,C,D).

Discussion

The results of this study show clearly that ultrasound covers and gels are embryo-toxic in an in-vitro mouse model and that...
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Figure 2. In-vitro toxicity test of pronucleate mouse ova cultured for 96 h in media containing different dilutions of a medium that was exposed for 1 h to cover A or B. Untreated human tubal fluid (HTF) medium was used as control (c).

Figure 3. In-vitro toxicity test of pronucleate mouse ova cultured for 96 h in media containing different dilutions of two sonographic carboxymethylene gels C and D or in media exposed to paraffin oil. cnt: continuous exposure. Untreated human tubal fluid (HTF) medium was used as control (c).

High dilutions are needed to restore normal cleavage and development in vitro.

There is no clear evidence that the vaginal use of materials that are severely embryotoxic in vitro has any clinical consequences in vivo. However, several studies on talc and starch dusting powders indicate that non-motile particles are able to migrate from the vagina to the oviducts and beyond. Some investigators (Wehner and Weller, 1986) could not detect translocated talc in the monkey uterus or beyond after several vaginal exposures to talc, but others (Egli and Newton, 1961) were able to recover inert carbon particles in human Fallopian tubes after 30 min of deposition in the vagina. Starch peritonitis caused by the translocation of starch on the surface of the condom through the Fallopian tubes into the peritoneum has been described (Saxen et al., 1963). It has been suggested (Kasper and Chandler, 1995) that talc on the surface of condoms can therefore cause Fallopian tube fibrosis and sclerosis and, therefore, total infertility. Furthermore, a rapid loss of sperm motility due to contamination of medium with sonographic gel at a concentration of 10% was demonstrated (Shimonovitz et al., 1994). Several studies have linked the use of talc and starch dusting powders on the lower abdomen and perineum with an increased risk of ovarian cancer (Henderson et al., 1971; Longo and You, 1979; Cramer et al., 1982;
Bernard et al., 1989; Chen et al., 1992). Physicians have been warned of the health risk and possible infertility risks to frequent condom-users (Kasper and Chandler, 1995), but many gynaecologists still use commercially available ultrasound covers and gels routinely during fertility treatment.

Based on the evidence reviewed above, it is clinically possible that condoms and gels used during ovarian stimulation cycles can impair the quality of ovulated oocytes, sperm cells and embryos. Furthermore, during oocyte aspiration (in IVF cycles), toxic compounds from the sonographic gel can be passed to ovaries or abdomen, with potential impairment of oocyte quality and fertilization. This is supported by our own clinical experience showing that follicular fluid from the first aspirated follicle during oocyte aspiration can be contaminated with paraffin oil droplets. Our observation suggests that intra-ovarian contamination with oils or gels used during oocyte aspiration may occur, as has previously been described during fine needle biopsies (Molyneux and Coghill, 1994).

Mouse embryos were used for ethical reasons and because of similarities in early preimplantation embryo development with the human (Hogan et al., 1986). Mouse and human embryos can be cultured in vitro to blastocysts in identical culture media (Scott et al., 1993; Van der Auwera, 1994). It is always difficult to extrapolate data from research on mice to the human situation. Furthermore, our study does not offer direct evidence for toxicity in vivo. However, we consider our observations on direct embryo toxicity in vitro to be clinically important and advocate the use of non-toxic paraffin oil and plastic polyethylene bags instead of commercially available gels and ultrasound latex covers during diagnostic and therapeutic procedures in reproductive medicine.

In conclusion our study has shown that two commercially available ultrasound latex covers and two sonographic carboxy-methylene gels are embryotoxic in an in-vitro mouse model. We recommend that vaginal ultrasonic measurements during infertility treatment should be performed using paraffin oil (as contact fluid) and plastic polyethylene bags (as ultrasound cover) since these products are not embryotoxic in vitro.

References