

Advances in quality control: mouse embryo morphokinetics are sensitive markers of *in vitro* stress

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STUDY QUESTION: Can time-lapse analysis of cell division timings [morphokinetics (MK)] in mouse embryos detect toxins at concentrations that do not affect blastocyst formation?

SUMMARY ANSWER: An MK algorithm enhances assay sensitivity while providing results 24–48 h sooner than the traditional mouse embryo assay (MEA).

WHAT IS KNOWN ALREADY: Current quality control testing methodology is sensitive but further improvements are needed to assure optimal culture conditions. MKs of embryo development may detect small variations in culture conditions.

STUDY DESIGN: Cross sectional—control versus treatment. Mouse embryo development kinetics of 466 embryos were analyzed according to exposure to various concentrations of toxins and toxic mineral oil.

MATERIALS, SETTING, METHODS: Cryopreserved 1-cell embryos from F1 hybrid mice were cultured with cumene hydroperoxide (CH) (0, 2, 4, 6 and 8 μ M) and Triton X-100 (TX-100; 0, 0.0008, 0.0012, 0.0016 and 0.002%). Using the Embryoscope, time-lapse images were obtained every 20 min for 120 h in seven focal planes. End-points were timing and pattern of cell division and embryo development. The blastocyst rate (BR) was defined as the percentage of embryos that developed to the expanded blastocyst stage within 96 h.

MAIN RESULTS AND THE ROLE OF CHANCE: BR was not affected for embryos cultured in the three lowest concentrations of CH and the four lowest concentrations of TX-100. In contrast, a unique MK model detected all concentrations tested ($P < 0.05$). The MK model identified toxicity in two lots of toxic mineral oil that did not affect BR ($P < 0.05$).

LIMITATIONS, REASONS FOR CAUTION: A limited number of toxins were used so that the results may not apply to all potential embryo toxins. A larger sample size may also demonstrate other statistically significant developmental kinetic parameters.

WIDER IMPLICATIONS OF THE FINDINGS: MKs in mouse embryos are a sensitive and efficient method for quality control testing of *in vitro* culture conditions. BR, the end-point of traditional quality control assays, did not detect sublethal concentrations of toxins in the culture milieu in our study. This study demonstrates that temporal variation at key developmental stages reflects the quality of the culture environment. An MEA that incorporates MK will provide enhanced sensitivity and faster turn-around times.

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Key words: time-lapse imaging / morphokinetics / embryo culture / quality control / toxicity

Introduction

The goal of an IVF program is a high live birth rate of healthy singletons. This success is dependent in part on a culture system that supports development of healthy embryos that are capable of realizing

their implantation potential (Puissant *et al.*, 1987; Hu *et al.*, 1998; Strandell *et al.*, 2000). Optimal culture conditions are the product of a good quality control system, which should maintain a stable culture environment and screen products for embryo-toxic substances (Gardner *et al.*, 2005; Lane *et al.*, 2008; Morbeck, 2012).

In order to optimize conditions and achieve consistent results, standardization and quality assessment of all culture components is essential. Bioassays employed by manufacturers and individual laboratories include the 1-cell and 2-cell mouse embryo assay (MEA) and the human sperm motility assay (HSMA). While the 1-cell MEA is more sensitive than the 2-cell MEA (Davidson *et al.*, 1988; Scott *et al.*, 1993; Hughes *et al.*, 2010; Morbeck *et al.*, 2010) or HSMA (Hughes *et al.*, 2010), it is unclear if the sensitivity of these assays is sufficient to detect toxins relevant to the human IVF laboratory. Strain-to-strain variation provides evidence that the current standard for QC testing, which uses the 1-cell FI hybrid mouse MEA, may not be sensitive enough to detect contaminants that affect human gametes and embryos (Khan *et al.*, 2013). Recent cases where toxic mineral oils passed MEA testing by the manufacturer but reached clinical laboratories and were subsequently recalled support this hypothesis (Morbeck, 2012; Morbeck *et al.*, 2012).

An inherent limitation of the MEA is the relative lack of sensitivity of the end-point: expanded blastocysts can form from few blastomeres and are not always associated with viability, a fact confirmed with a more objective end-point such as cell number (Lane and Gardner 1997). In contrast, time-lapse imaging is a continuous, objective record of embryo development that provides timing of cell division events (Gonzales *et al.*, 1995; Arav *et al.*, 2008; Lemmen *et al.*, 2008). While timing of cell divisions has been promoted as an important variable for selecting embryos for transfer in the clinical IVF laboratory (Pribenszky *et al.*, 2010; Wong *et al.*, 2010; Azzarello *et al.*, 2012; Cruz *et al.*, 2012; Hlinka *et al.*, 2012; Kirkegaard *et al.*, 2012; Meseguer *et al.*, 2012), timing as a QC tool in the MEA has not been adopted, likely due to limited evidence and lack of standardization. The development of

time-lapse imaging technology provides a tool to test the hypothesis that timing of cleavage events is a sensitive marker for *in vitro* stress.

The aim of this study was to determine whether toxins at concentrations that do not affect blastocyst formation could be detected by morphokinetic (MK) analysis of cell division. The relative sensitivity of MKs was tested with two toxins to develop a quality control algorithm based on timing of cell divisions. These markers were then applied to mineral oil that passed standard quality control testing but had been recalled by the manufacturer due to potential toxicity to human embryos.

Materials and Methods

Culture of embryos

Cryopreserved 1-cell mouse embryos from FI hybrid mice were obtained from Embryotech Laboratories (Haverhill, MA, USA). The embryos were thawed according to the manufacturer's instructions and allowed to equilibrate for 10 min at room temperature in HTF with HEPES (HTF-HEPES; Irvine Scientific, Irvine, CA, USA) containing 0.1 mg/ml polyvinyl alcohol (PVA) (Sigma Chemical, St. Louis, MO, USA). Following equilibration, embryos were placed in individual wells ($n = 10$ /treatment per replicate) in an EmbryoSlide (Unisense Fertilitech, Aarhus, Denmark) and placed immediately into an EmbryoScope (Unisense Fertilitech) for time-lapse analysis. All tests were performed with HTF containing EDTA and glutamine (In Vitro Care, Frederick, MD, USA) supplemented with 0.1 mg/ml PVA. EmbryoSlides were prepared the day before thawing embryos and were equilibrated overnight in an incubator with 5% O₂, 6.5% CO₂ and balance nitrogen. Individual wells in the EmbryoSlide contained either 10 μ l (for oil toxicity experiments) or 25 μ l (for Triton X-100) HTF and the wells were covered with 1.2 ml mineral oil (Fisher Scientific, Pittsburgh, PA, USA).

Table 1 Division kinetics of 1-cell mouse embryos cultured with increasing concentrations of CH.

Embryos	CH				
	0 μ M	2 μ M	4 μ M	6 μ M	8 μ M
	31	31	30	32	29
t2 (h)	5.8 (5.3–6.3)	5.7 (5.0–6.4)	6.1 (5.5–6.7)	5.6 (5.0–6.3)	6.7 (5.9–7.6)
t3 (h)	28.2 (27.3–29.1)	27.3 (25.4–29.2)	30.7 (29.1–32.3)	30.9 (29.5–32.2)*	33.4 (31.8–34.9)***
t4 (h)	29.0 (28.0–29.9)	29.0 (27.1–30.9)	33.0 (30.9–35.1)*	32.5 (30.7–34.4)**	34.4 (32.8–36.1)***
t5 (h)	38.3 (37.1–39.4)	39.9 (38.5–41.2)	44.4 (42.1–46.7)***	45.8 (44.1–47.5)***	49.7 (46.9–52.4)***
t6 (h)	38.8 (37.7–39.9)	40.5 (39.1–41.9)	45.8 (42.6–49.0)***	46.8 (44.8–48.7)***	51.1 (48.2–54.0)***
t7 (h)	39.5 (38.6340.7)	42.0 (40.2–43.8)	46.0 (43.7–48.2)***	47.4 (45.5–49.3)***	53.1 (49.7–56.5)***
t8 (h)	40.2 (38.9–41.5)	43.1 (40.8–45.4)	46.6 (44.3–48.9)***	48.0 (46.0–50.0)***	54.0 (50.3–57.6)***
tM (h)	52.8 (51.1–54.5)	55.9 (54.4–57.5)*	58.6 (56.7–60.6)***	59.5 (57.5–61.5)***	68.2 (61.6–74.8)***
tB (h)	67.1 (64.9–69.3)	70.0 (67.5–71.6)	73.6 (70.6–76.5)**	74.9 (71.6–78.2)***	90.6 (90.6–90.6)**
tEB (h)	75.7 (73.8–77.6)	79.7 (77.2–82.2)	84.0 (80.7–87.3)***	85.9 (82.1–89.7)***	
cc2 (h)	22.4 (21.7–23.1)	21.6 (19.8–23.4)	24.6 (23.2–26.0)	25.3 (24.0–26.5)**	26.7 (25.3–28.1)***
s2 (h)	0.8 (0.6–1.1)	1.7 (0.7–2.7)	2.3 (1.3–3.4)*	1.6 (.6–2.6)	1.3 (0.8–1.8)
i3 (h)	9.3 (8.8–9.7)	10.8 (9.1–12.6)	11.4 (9.5–13.2)	13.3 (11.9–14.6)***	16.7 (14.4–18.9)***
s3 (h)	1.9 (1.4–2.4)	3.2 (1.9–4.5)	3.4 (2.4–4.4)	2.7 (2.1–3.3)	4.4 (2.5–6.3)***

Values are mean (95% CI). t2, t3, t4, t5, t6, t7 and t8, cleavage times from a zygote to a 2–8-cell embryo; tM, time to morula formation; tB, time to formation of blastocoel cavity; tEB, time to expanded blastocyst; cc2, duration of 2-cell stage (t3–t2); i3, duration of 4-cell stage (t5–t4); s2, synchrony second cell cycle (t4–t3); s3, synchrony of third cell cycle (t8–t5). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (Dunnett's test).

Table II Division kinetics of I-cell mouse embryos cultured with increasing concentrations of Triton X-100 (TX-100).

Embryos	Triton X-100					
	0	0.0004%	0.0008%	0.0012%	0.0016%	0.0020%
	29	31	32	31	32	32
t2 (h)	5.6 (4.9–6.3)	6.6 (5.8–7.4)	6.5 (5.8–7.2)	6.8 (6.2–7.4)	7.4 (6.6–8.1)**	7.9 (7.0–8.7)***
t3 (h)	27.3 (26.4–28.2)	27.8 (26.1–29.5)	29.2 (28.0–30.4)	30.0 (29.2–30.8)**	31.4 (30.4–32.4)***	33.5 (32.5–34.6)***
t4 (h)	28.2 (27.2–29.2)	29.7 (27.6–31.7)	30.7 (29.6–31.9)*	30.9 (30.0–31.8)*	32.6 (31.5–33.7)***	35.5 (33.8–37.2)***
t5 (h)	36.8 (35.7–37.8)	38.1 (36.7–39.6)	39.0 (37.7–40.2)	39.5 (38.5–40.4)*	41.4 (40.3–42.3)***	45.0 (43.2–46.7)***
t6 (h)	37.2 (36.1–38.3)	38.7 (37.1–40.2)	39.5 (38.3–40.8)	40.0 (39.0–41.0)*	41.9 (40.7–43.1)***	45.7 (43.9–47.4)***
t7 (h)	37.9 (36.7–39.0)	40.3 (38.3–42.2)	40.9 (39.3–42.5)*	40.9 (39.8–42.0)*	42.9 (41.5–44.3)***	46.6 (45.1–48.1)***
t8 (h)	38.3 (37.1–39.4)	40.3 (38.6–42.0)	41.5 (40.0–43.1)**	41.6 (40.4–42.8)**	43.5 (42.0–45.0)***	47.5 (45.8–49.1)***
tM (h)	51.2 (49.6–52.8)	54.2 (52.2–56.3)	56.3 (54.4–58.3)	56.2 (54.7–57.6)***	60.4 (58.4–62.3)***	66.1 (63.9–68.3)***
tB (h)	63.9 (62.3–65.6)	68.7 (65.9–71.5)*	72.3 (69.3–75.3)***	72.3 (70.3–74.4)***	78.9 (76.2–81.5)***	84.0 (81.4–86.4)***
tEB (h)	75.7 (72.7–78.7)	80.6 (80.0–84.2)	83.4 (79.9–86.9)**	85.6 (82.2–89.1)***	89.2 (85.9–92.5)***	95.8 (92.0–99.5)***
cc2 (h)	21.7 (21.1–22.3)	21.2 (19.8–22.6)	22.7 (21.8–23.5)	23.2 (22.7–23.7)*	24.0 (23.5–24.6)***	25.7 (25.1–26.2)***
s2 (h)	0.9 (0.6–1.1)	1.8 (0.8–2.9)	1.6 (0.7–2.4)	0.9 (0.6–1.1)	1.2 (0.9–1.5)	2.0 (1.1–2.8)
i3 (h)	8.6 (8.2–8.9)	8.5 (7.4–9.6)	8.2 (7.8–8.7)	8.5 (8.1–9.0)	8.8 (8.5–9.1)	9.5 (8.6–10.3)
s3 (h)	1.5 (1.1–1.9)	2.4 (1.8–3.0)	2.6 (1.8–3.4)	2.1 (1.7–2.6)	2.1 (1.5–2.7)	3.1 (2.3–3.9)

Values are mean (95% CI). t2, t3, t4, t5, t6, t7 and t8, cleavage times from a zygote to 2–8-cell embryo; tM, time to morula formation; tB, time to formation of blastocoel cavity; tEB, time to expanded blastocyst; cc2, duration of 2-cell stage (t3–t2); i3, duration of 4-cell stage (t5–t4); s2, synchrony second cell cycle (t4–t3); s3, synchrony of third cell cycle (t8–t5). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Experiment one: cumene hydroperoxide

Cumene hydroperoxide (CH; Sigma-Aldrich) was used as a surrogate for peroxides that can accumulate naturally in mineral oil (Otsuki et al., 2007; Morbeck et al., 2010) and was prepared as previously described (Hughes et al., 2010). For each replicate ($n = 3$), an EmbryoSlide ($n = 5$ /replicate) was prepared for each concentration of CH (0, 2, 4, 6 or 8 μM).

Experiment two: Triton X-100

Triton X-100 (TX-100) is a detergent that was previously identified as a contaminant of mineral oil (Morbeck et al., 2010). TX-100 was added directly to HTF and embryos were cultured in 25 μl in individual wells in an EmbryoSlide covered with 1.2 ml of mineral oil. For each replicate ($n = 3$), an EmbryoSlide ($n = 6$ /replicate) was prepared for each concentration of TX-100 (0, 0.0004, 0.0008, 0.0012, 0.0016 or 0.002% v/v).

Experiment three: recalled oil lots

Two lots of US Pharmaceutical grade mineral oil that were recalled in 2010 due to suspicion of toxicity during human embryo culture were compared with control oil (Fisher). The two lots of oil (Lot 1 and Lot 2) were from different suppliers and passed standard mouse embryo testing. A sample size estimate was performed to determine the number of embryos needed to detect a difference using the MK model based on results from experiments 1 and 2. Assuming 60% of embryos in the control group fit the MK model, 38 embryos in each group would be needed to detect a decrease in the model fit rate to 40% (20% decrease) with 80% power and an α -error rate of 0.05. Therefore, four replicates ($n = 40$ embryos/treatment) were performed with 10 embryos per replicate in HTF.

Time-lapse system

Precise division kinetics were captured using time-lapse microscopy with an EmbryoScope incubator (Unisense Fertilitech). The embryos were

placed in the incubator and seven planes of images were obtained every 20 min for 120 h.

The blastocyst rate (BR) was defined as the percentage of embryos that developed to the expanded blastocyst stage by 96 h. Cell division timings were determined manually and included t2, t3, t4, t5, t6, t7 and t8 (time from thawing to 2, 3, 4, 5, 6, 7 and 8 cells), time of compaction, tM, time to formation of a blastocoel, tB and time to an expanded blastocyst (tEB). Other parameters included cc2 (duration of the second cell cycle—the time an embryo is at the 2-cell stage), i3 (duration of the third interphase—the time an embryo is at the 4-cell stage), s2 (synchrony of the second cell division, which is the time an embryo is at the 3-cell stage) and s3 (synchrony of the third cell division, which is the time an embryo contains 5, 6 or 7 cells).

Data and statistical analysis

Data were analyzed using a one-way analysis of variance with Dunnett's test for comparison of timings to the control and the χ^2 test for comparison of proportions. The timings followed a mostly normal distribution. Timings that showed significant treatment effects were used to develop a multivariate model. Optimal timing for these events (t5, cc2, i3 and tB) was defined as less than the upper 95% confidence interval (CI) of the control for each replicate. Statistical analyses were performed using JMP statistical software (SAS Institute, Cary, NC, USA).

Results

Toxin sensitivity

Timing of cleavage events, cleavage synchrony and cell cycle length for different concentrations of CH and TX-100 are presented in Tables I and II, respectively. The completion of the second cell cycle (t4) was the earliest event with a delay in response to CH at 4 μM , with a more

profound effect at initiation of the third cell cycle (t5). The synchrony of the third cell cycle (s3) was not affected by CH at this concentration, indicating that the timings of t6, t7 and t8 are unchanged despite effects on t5 and thus do not provide additional information. CH had a strong effect on time of morula formation and tEB at 4 μM, though these variables are the least objective and most difficult to standardize. In contrast, blastocoel formation (tB), a feature of development that is distinct and easily assessed, was delayed at 4 μM CH. Duration of the second and third cell cycles was affected by CH at 6 μM.

Formation of a blastocoel cavity (tB) was very sensitive to TX-100 (Table II), with a delay of nearly 5 h at the lowest concentration tested (0.0004%; $P < 0.05$). Early cleavage events were not consistently affected by TX-100 at the two lowest concentrations tested. However, an effect of treatment was observed at 0.0012% TX-100 on most of the early timings, including t5 and cc2.

Based on these results, the percentage of embryos that exhibited optimal timings based on the 95% CI for the control for each replicate were compared across concentrations within treatment for t5, cc2 and tB (Fig. 1). Optimal timings relative to the control's 95% CI

provides an objective standardization per replicate that accounts for timing variation among replicates that is inherent with the MEA. Individually, optimal timings for the three variables detected all but the lowest concentration of CH tested (Fig. 1A). In contrast, tB but not t5 and cc2 detected the two lowest concentrations of TX-100 (Fig. 1B), illustrating a difference in the effect of these two toxins on early versus late stages of embryo development.

A powerful feature of time-lapse technology is the ability to combine several objective measures into one analysis model. In this phase of the study, t5, cc2 and tB were combined into one optimized model using the control group's 95% CI (MK Model), a model that provides results by 72 h of culture. In this model, the lowest concentration of both CH (Fig. 2A) and TX-100 (Fig. 2B) were detected by MKs. In contrast, the traditional end-point of expanded BR at 96 h was able to detect only the highest concentrations of either toxin (Fig. 2A and B).

Mineral oil for IVF

Timing of cleavage events, cleavage synchrony and cell cycle length for control, Lot 1 and Lot 2 oils are presented in Table III. Similar to CH, the initiation of the third cell cycle (t5) was affected by the two suspect oil lots. In contrast to both CH and TX-100, duration of the third interphase (i3) was longer ($P < 0.001$) for both oils, indicating an effect on mitosis at this stage of development that was not evident in the toxin studies. When compared with the control's 95% upper CI, fewer embryos met the criteria for t5 and i3 in the two lots of

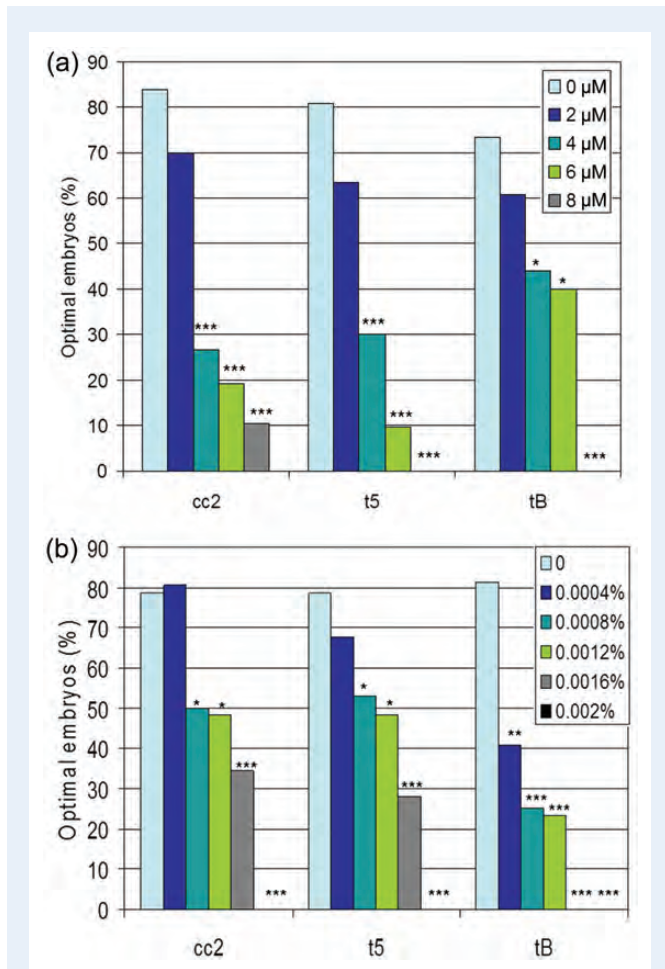


Figure 1 Percentage of embryos meeting optimal criteria (<95% CI of controls) for cc2, t5 and tB in response to (a) CH or (b) Triton X-100. t5 = time to 5-cell embryo; cc2 = second cell cycle length; tB = time to formation of blastocoel cavity * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus the control (χ^2).

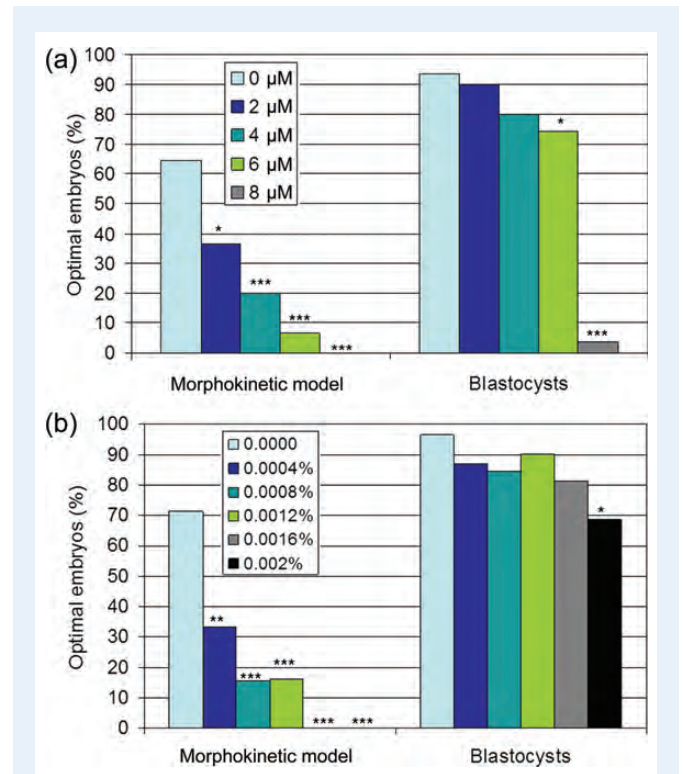


Figure 2 Percentage of embryos meeting optimal criteria (<95% CI of controls) for the morphokinetic (MK) model or BR in response to (a) CH or (b) Triton X-100. MK Model = combined optimal t5, cc2, tB; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus the control (χ^2).

Table III Division kinetics of I-cell mouse embryos cultured with different lots of mineral oil.

Embryos	Control	Lot 1	Lot 2
	42	42	42
t2 (h)	10.2 (9.8–10.7)	10.7 (10.3–11.1)	11.0 (9.9–12.2)
t3 (h)	34.1 (33.4–34.7)	35.6 (34.8–36.4)*	35.2 (34.2–36.2)
t4 (h)	34.9 (34.2–35.6)	36.6 (35.7–37.5)*	35.9 (34.8–37.0)
t5 (h)	44.7 (43.8–45.5)	48.4 (47.3–49.5)***	47.4 (45.7–49.1)***
t8 (h)	47.9 (46.6–49.3)	51.8 (50.3–53.2)**	49.7 (47.6–51.8)
tM (h)	59.7 (58.1–61.2)	65.4 (64.0–66.9)***	62.0 (60.1–63.9)
tB (h)	73.1 (71.0–75.2)	77.6 (75.1–80.1)*	76.9 (73.7–80.1)
tEB (h)	91.1 (88.4–93.7)	97.1 (94.4–99.9)**	94.3 (92.0–96.6)
cc2 (h)	23.8 (23.4–24.2)	24.9 (24.3–25.4)	24.2 (22.8–25.6)
s2 (h)	0.8 (0.6–1.1)	1.0 (0.7–1.3)	0.7 (.5–1.0)
i3 (h)	9.8 (9.4–10.2)	11.8 (11.1–12.4)***	11.4 (10.6–12.2)***
s3 (h)	3.3 (2.4–4.1)	3.4 (2.7–4.0)	2.8 (2.2–3.4)

Values are mean (95% CI). t2, t3, t4, t5, t6, t7 and t8, cleavage times from a zygote to 2–8-cell embryo; tM, time to morula formation; tB, time to formation of blastocoel cavity; tEB, time to expanded blastocyst; cc2, duration of 2-cell stage (t3–t2); i3, duration of 4-cell stage (t5–t4); s2, synchrony second cell cycle (t4–t3); s3, synchrony of third cell cycle (t8–t5). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (Dunnett's test).

affected oil, whereas cc2 detected a difference only for Lot 1 and tB only for Lot 2 (Fig. 3A).

When a new model was developed that included the four variables identified in both studies (t5, cc2, i3 and tB), an adverse effect of both Lot 1 and Lot 2 was identified (Fig. 3B; $P < 0.05$). Similar to results of manufacturer's quality control testing, neither of these lots affected BRs and all three lots would be considered acceptable with rates $>80\%$.

Discussion

In this study we demonstrated that timing of early cleavage events of mouse embryos identified with time-lapse imaging are sensitive markers of toxins at concentrations that do not affect blastocyst development, the primary end-point of standard quality control testing. The sensitivity of MK analysis was confirmed when it successfully identified mineral oil that had passed manufacturer's quality control testing but was recalled due to questions of toxicity to human embryos. In addition to improved sensitivity over the 96 h I-cell MEA, the cell cycle events that were most sensitive to toxicity occurred within the first 72 h of culture. These results present a new paradigm for quality control testing: a subjective MEA with improved sensitivity that provides results at least 24 h sooner than the standard MEA.

While time-lapse analysis of embryo development has been used in research (Bavister, 1995) and was recently introduced into clinical IVF (Lemmen et al., 2008; Basile et al., 2013), this is the first study of the utility of time-lapse for quality control of products used in the IVF laboratory. Previous work with animal models provide evidence that timing of cleavage events could be useful for determining viability of embryos, and therefore may provide a tool for quality control (McKiernan and Bavister 1994; Gardner et al., 2005; Taft 2008). Additional studies indicated that the duration of the third cell cycle

(4–8 cells) showed a significant correlation with blastocyst formation for hamster embryos (Gonzales et al., 1995). In contrast, timing of the first cell division (Arav et al., 2008; Pribenszky et al., 2010) and the duration of the second cell cycle (Pribenszky et al., 2010) in mouse embryos were correlated with blastocyst formation *in vitro*. While species differences exist and these may depend on timing of activation of the embryonic genome, the timing of the first three cell divisions is dynamic and reflects the developmental capacity of the early embryo. Unlike blastocyst formation, which is known to be a poor measure of viability (Lane and Gardner 1996, 1997), our results demonstrate that time-lapse imaging provides subjective timings of distinct developmental events that are responsive to quality of culture conditions.

Development of a bioassay with sensitivity similar to a human embryo is fraught with challenges. Few studies have compared the sensitivities of the various bioassays reported in the literature, and a direct comparison of toxin sensitivity with human embryos and toxins like TX-100 and CH has not been performed. Though a direct comparison of bioassays to human embryos is unlikely, validation of bioassay sensitivity using a robust scientific method is critical to assure that manufacturers and IVF laboratories are using the most sensitive and appropriate assays available. Most studies report toxicity of individual laboratory items without an identification of the toxin (Naz et al., 1986; Fleming et al., 1987; Claassens et al., 2000); however, reports with concentrations of known or suspected toxins have consistently demonstrated that the I-cell MEA is superior to the 2-cell MEA or the sperm survival assay (Davidson et al., 1988; Scott et al., 1993; Hughes et al., 2010; Morbeck et al., 2010). Even though the I-cell MEA is the industry standard, mineral oil that passed manufacturer QC illustrates that the assay, with BR as an end-point, can fail to detect clinically relevant toxins. This relative insensitivity of the MEA may be strain dependent, and at least one manufacturer uses an outbred mouse strain in their QC program.

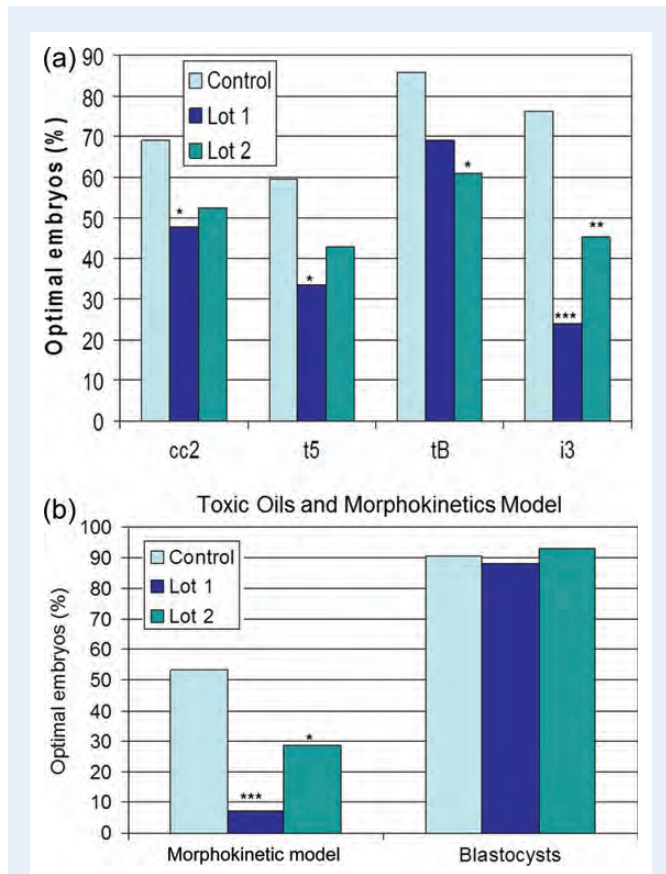


Figure 3 Percentage of embryos meeting optimal criteria (<95% CI of controls) for (a) individual parameters or (b) a morphokinetic (MK) model or BR for two lots of embryotoxic mineral oil (Lot 1 and Lot 2). t5 = time to 5-cell embryo; cc2 = second cell cycle length; tB = time to formation of blastocoel cavity; i3 = time at 4-cell stage; MK Model = combined optimal t5, cc2, tB and cc3; *P < 0.05, **P < 0.01, ***P < 0.001 versus the control oil (χ^2).

Using the same toxins (TX-100 and CH), we have confirmed that embryos from outbred mice are significantly more sensitive to *in vitro* culture stress than either inbred or F1/hybrid embryo (Khan *et al.*, 2013). Embryos from outbred mice were four times more sensitive to CH than other strains and detected the lowest concentration tested (2 μ M), a concentration that was also detected by the MK model in this study. The MK model also detected TX-100 at concentrations similar to and lower than the concentration detected by outbred mouse embryos. Application of the MK model to an outbred MEA may provide even further sensitivity, though there are limitations inherent with outbred mouse embryos that have limited their use as a routine QC tool.

The importance of an optimal environment for IVF and preimplantation embryo development cannot be overstated. Recent reports of adverse effects of mineral oil on pregnancy rates illustrate the importance of effective supply management in the IVF laboratory (Morbeck, 2012). Beyond the immediate consequences of suboptimal culture are the concerns with long-term health of children conceived through IVF. Several animal models have illuminated a direct role of culture environment on many facets of embryo viability and long-term

development. For instance, mouse embryos cultured under suboptimal conditions demonstrated differences in birthweight (Banrezes *et al.*, 2011), imprinting (Fernandez-Gonzalez *et al.*, 2004; Rivera *et al.*, 2008) and gene expression (Ecker *et al.*, 2004; Rinaudo and Schultz 2004; Giritharan *et al.*, 2007). Errors in imprinting in domestic animals have been linked to culture conditions as well (Lonergan *et al.*, 2003). Adverse effects of *in vitro* culture may also affect human development, with evidence that shorter culture times yield better long-term outcomes (Kallen *et al.*, 2010; Kalra *et al.*, 2012) and that culture media composition can affect birthweights (Dumoulin *et al.*, 2010; Nelissen *et al.*, 2012).

The results of these studies provide a new approach to quality control testing for products used in the clinical IVF laboratory. A desirable quality control method should be reproducible, sensitive and require a minimum investment of time and labor. An MEA with MKs incorporates these elements and with the prospect of automated cell division annotation and data analysis will likely provide an even more robust and effective quality control tool for both manufacturers and IVF laboratories.

Authors' roles

H.S.W., J.R.F. and D.L.W. performed the experiments and collected the data. D.E.M. designed the study and analyzed the data with input from H.S.W. H.S.W. wrote the manuscript with input from D.E.M.

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Conflict of interest

None declared.

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