# Changing old trends in IVF laboratory cleaning and disinfection

# Aims

- To evaluate efficacy of 6% hydrogen peroxide, 70% ethanol and Oosafe® using differential bacterial counts
- 2) To evaluate safety of the three products on mouse embryonic development

# Method

## Differential bacterial counts

Samples were obtained using  $\text{Difco}^{\text{TM}}$ Hycheck<sup>TM</sup> non-selective agar slides. Replicates were taken from various locations in a student embryology laboratory.

## Mouse embryo testing

Ethical approval was obtained from Monash Medical Centre Animal Ethics Committee. At the two-cell stage, embryos were exposed to:

- 1) Direct disinfectant contact
- 2) Disinfectant residue
- Media equilibrated in MINC<sup>™</sup> incubators recently cleaned by wiping with disinfectant or water (control). Blastocyst formation at 72 h was the experiment end point.

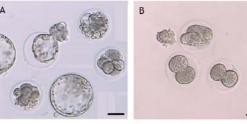
#### Sperm toxicity testing

Semen samples with 90% motility were analysed after a 2h incubation in disinfectant cleaned chambers.

# Results

| Differential bacterial counts |   |
|-------------------------------|---|
| Treatment group               | Average colony growth<br>per Difco <sup>™</sup> side (24 h) |
| Control                       | 13.0 ± 1.0 <sup>a</sup>                                     |
| Dry wipe                      | $0.3 \pm 0.3^{b}$   |
| 6% hydrogen peroxide          | 0.0 <sup>b</sup>  |
| Oosafe®                       | 0.0 <sup>b</sup>  |
| 70% ethanol                   | $0.3 \pm 0.3^{b}$   |

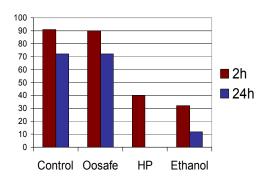
\*Different superscripts within the same column indicate significance difference (P<0.05).



# Mouse embryo testing

- Direct disinfectant contact and residue inhibited embryo growth at the 2-cell stage in all cases.
- 92% of embryos incubated in the Oosafe® cleaned MINC<sup>™</sup> reached blastocyst stage, similar to control levels giving 67% blastocyst development (Fig. 1A,C).
- 70% ethanol and 6% hydrogen peroxide cleaning inhibited growth at the 2-cell stage (Fig. 1B,D).

#### Sperm toxicity testing (% motility)

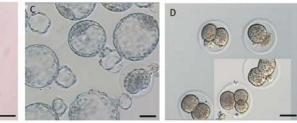


# Significance

Embryos are extremely sensitive to disinfectant residue and fumes. There is no completely safe option and all IVF laboratories are encouraged to re-evaluate their cleaning protocols.

This study indicates that Oosafe® poses the least risk to embryo development and sperm viability. Ethanol and hydrogen peroxide both had a detrimental effect on embryos and sperm, but could still be used for general laboratory cleaning. A combination of disinfectants might be ideal for effective cleaning protocols.

#### Great care must be taken so that embryo growth and subsequent pregnancy rates are not affected by essential cleaning protocols.



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Figure 1 72 h incubation in disinfected MINC<sup>™</sup>. Control (A), 6% hydrogen peroxide (B), Oosafe® (C) and 70% ethanol (D) treatment. Scale bar: 50 µm.

70% ethanol is the most commonly used IVF laboratory disinfectant. This is a concern, as alcohol based disinfectants release VOCs which are directly toxic to embryos.

BACKGROUND

the success of IVF.

Air quality is crucial for

Hydrogen peroxide is a potential alternative cleaning product, which decomposes to water and oxygen.

#### Oosafe® is a

commercially available product that contains quaternary ammonium compounds and no VOCs.

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