ORIGINAL ARTICLE

Toxic town: ART disinfectants damage human sperms: An investigation into potential toxicity of ART disinfectants on human sperms

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ABSTRACT

Aim: To investigate toxicity of disinfectants using human sperm motility assay (HSMA) in order to establish universal disinfection protocol, safe by all means for ART set up.

Methods: Semen samples obtained from normospermic men and processed through density gradient, pellet was resuspended in 5ml of KSOM handling medium and motility assessed at 0 hours. The replicate splitted equally into five 1ml aliquots.In *direct contact trial,* four test tubes treated by adding 0.5ml of each disinfectant simultaneously (70% Ethanol, Fertisafe, Fertisafe Plus & Farmacidal) directly, one test tube without disinfectant being a control.

Results: Human sperm motility was lost after 24hrs of *direct contact trial* treatment with disinfectants as compared to control ($p<0.01$). Sperm motility lost was more than 90 percent after 24hrs of *direct contacttrial* of disinfectants.

Conclusion: Significant association of disinfectant use in ART setup and loss of sperm motility was found in *direct contacttrial.*

Keywords: Normospermic, Toxicity, Disinfectant, Sperm motility, assistedreproductive technologies (ART)

INTRODUCTION

One of the fundamental and critical perspectives of ART is maintenance and viability of reproductive cells i.e., gametes and embryos in an ideal laboratory environment where reproductive cells are cultured *in vitro*, despite of the fact that to date the evidence for the optimal *IVF* practice has been limited. Notwithstanding, the utmost factor in the success of ART is the quality of laboratory air[1].

In the course of last decade studies have demonstrated the negative impact of poor air quality because of air borne toxicants, for example, Volatile Organic Compounds– VOCs etc., on embryo advancement and implantation potential[2,3,4]nonetheless, with better air quality showed increased *in vitro* fertilisation outcome[5,6]. It is significant to keep up strict culture conditions for controlling developing moieties and gametes in ART and it is compulsory to do each and every piece of this technique with full cleanliness and under aseptic conditions[7]. To sterilise the Laboratory air, disinfectants are used which are reagents that take out most of bothersome microorganisms with the exception of bacterial spores[8] killing and controlling microorganisms is the advantage and embryo toxicity is perhaps the pitfall of disinfectants[9]. To date there is no strict laboratory decontamination protocol to follow in ART setup. The best disinfectant would be the one with best "biocidal" and "biostatic" action, diminishing gametes and embryobioburden with minimum cytotoxicity, presenting wellbeing for working embryologists in lab.

Oosafe Spar MED and Fermacydal industrially manufactured disinfectants short while ago, evolved over the previous years, VOC free[10] contain quaternary wide range of antimicrobial property with a superb surface cleaning activities[11]. Being membrane active reagents QACs follow up on cytoplasmic layers disturbing typical cell morphology and subsequently creating harming impacts to microorganisms integrity and structure[12].Likewise they attack intracellular targets by building linkages with DNA[13].Fertisafe and Fertisafe Plus in addition to foresaid disinfectants are chlorine and silver dihydrogen citrate containing commercial disinfectants, basically VOC and QAC free, acknowledged to be embryo safe and sperm safe[14].

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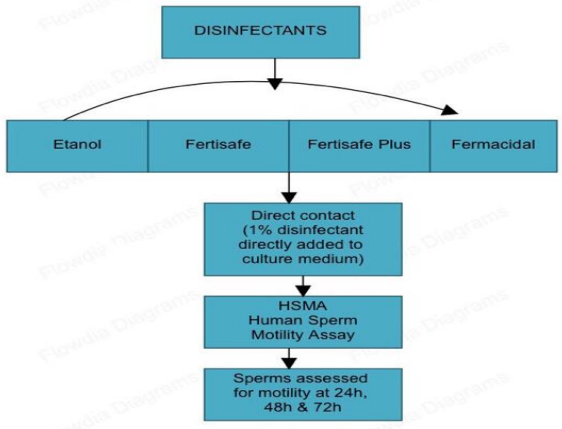
MATERIAL AND METHOD

It was a quantitative experimental study, HSMA (Human sperm motility assay). We used four different disinfectants in our experiments as (a) Ethanol, (b)Fertisafe (SDC) , (c) Fertisafe Plus (SDC) , (d) Fermacidal (IC Products)
We generated experimental conditions in order to test disinfectants toxicity on human sperms in *direct contact trial.*

Fig. 1: Study design showing direct contact of disinfectants with culture medium containing Sperm except control.



Human sperm motility assay: Willing normospermic males were approached and semen samples were taken; we collected one replicate from each person. After obtaining sperm samples were assessed and centrifuged through density gradient, diluted in 5ml KSOM handling medium and total motility (PR+NP) was calculated by counting 200 spermatozoa at five different fields to avoid sampling error in each replicate (WHO Laboratory Manual)at 0 hour. For each treatment condition, we divided

sperm aliquots equally in 5ml tubes, two replicates were produced for each condition. Sperm motility was assessed using WHO Laboratory Manual after 24hrs, 48hrs and 72hrs and loss of sperm motility was formulated by comparing sperm motility at 0 hr. The treatment conditions were created with disinfectants in *direct contact* after diluting in KSOM handling medium at 1%v/v concentration in comparison with control without disinfectant. Experiment was completed twice with two different samples of sperm from two individuals. We treated HSMA with *direct contact* as shown in figure 1.

All experimental procedures were conducted in accordance with the ethical guidelines established and approved by the Monash Medical Centre Ethics Committee (Ethics Number: A11/84)The study was carried out in Monash Medical centre, Monash university, Australia in 2015.

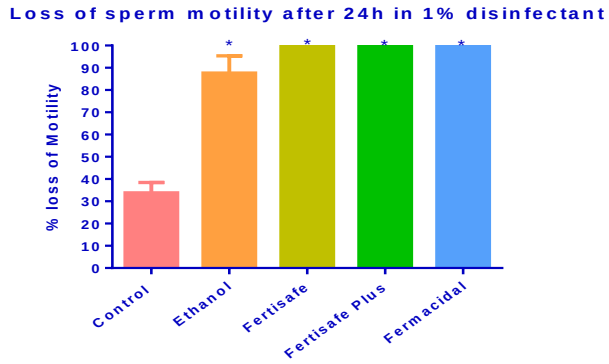
PH analysis of disinfectant in culture medium: Equilibrated KSOM culture medium was prepared as described by Summers et al., 1995. Culture medium was separated into 5mL test tubes with 1% disinfectant v/v applied. Two replicates were prepared for each disinfectant, plus two for the control. PH was measured using the pH meter PHM210 standard pH meter (Radiometer Analytical, Villeurbanne, Lyon, France).

Statistical Analysis: For the sperm tests, Dunnett's multiple comparison post-hoc test compared to control was used.

RESULTS

HUMAN SPERM MOTILITY ASSAY: At time 0 the average sperm motility was 76% +/- 4.7% (Table 1). Across the 4 replicates a total loss of motility was observed in the Fermacidal, Fertisafe and Fertisafe Plus treatment groups. Ethanol showed a total loss of motility in 3 of the 4 replicates and a drop to 37.6% +/- 19.5% motility in one replicate. Overall there was a significantly higher loss of motility in all treatment groups in relation to the control (Figure 2). In the Fertisafe Plus treatment group there was agglutination of the sperm observed across all replicates. Comparatively, Ethanol was only considered safe for sperm when directly applied into the medium (Table 1).

Fig. 2. Percentage of the loss of sperm motility when exposed to 1% v/v disinfectant in direct contact after 24 hours.



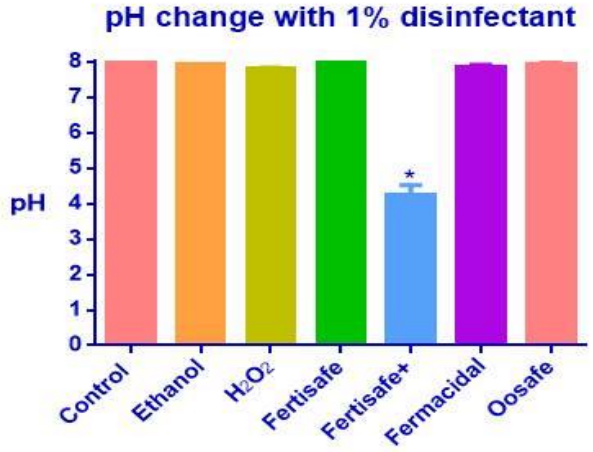
*In comparison to control statistically significant (p<0.05)

Table 1. Human sperm motility– loss of motility in 1% v/v of disinfectants

Groups	**Loss of Motility (%)**	**P-value**
Control	33.79 ± 4.70	-
70 % Ethanol	87.61 ± 7.80	0.0071**
Fermacidal	100.00	0.0059**
Fertisafe	100.00	0.0059**
Fertisafe Plus	100.00	0.0059**
Control	33.79 ± 4.70	-

PH analysis of disinfectant: During the experiment, it was observed that Fertisafe Plus would react with the phenol red in the KSOM culture media and change its complexion to yellow.Therefore, all the disinfectants were assessed to determine if they had an effect on the pH. As previously observed, Fertisafe Plus did change the colour of phenol red, indicating an acidic effect on the medium. This was quantified as an average pH of 4.255 +/- 0.265, which was statistically significant to the control (Table 2). All other disinfectants showed minute decreases in pH that were not statistically significant to the control.

Fig.2: The effect of 1% disinfectant on pH of culture media (v/v).



*In comparison to control statistically significant (p<0.05).

Table 2. Effect of pH changes of 1% disinfectant when compared to the control

Groups	**Average pH (+/- SEM)**	**P-value**
Control	7.955 +/- 0.015	
70 % Ethanol	7.92 +/- 0.01	0.9997
3% Hydrogen Peroxide	7.82 +/- 0.01	0.8683
Oosafe	7.93 +/- 0.08	0.9997
Fertisafe	7.955 +/- 0.015	>0.9999
Fertisafe Plus	4.255 +/- 0.265	<0.0001
Fermacidal	7.865 +/- 0.045	0.9723

DISCUSSION

Disinfection is a typical practice in ART facilities however unluckily to date, there is an absence of consensus on cleaning protocol. Nevertheless, the period of disinfectants toxicity evolved with the comprehension of connection between different disinfectants being used and decrease in development of developing cells in IVF[15,16]. We researched disinfectants' toxic quality utilizing HSMA (Human Sperm motility assay).

HSMA (human sperm motility assay) when treated with disinfectants in ***direct contact*** showed loss of total motility after 24hrs when compared to control.

Fermaicdal is VOC free quaternary ammonium compound (QACs) containing disinfectant proved safest to be around gametes supported by one study in past that demonstrated absence of gamete toxicity with QACs[17].During experimental investigation, the loss of Sperm motility was 100% after 24hr of *direct contact* demonstrating extraordinary Sperm senstivity with Fermacidal bolstered by a past research that revealed QACs cause embryo toxicity in mice[17] and fruitfulness

problems in grown-up mice[18] albeit further research is required in this regard.

Fertisafe Plus and Fertisafe both demonstrated to have potential toxicities in direct contact with sperms. The experimental conditions we created, sperms demonstrated outrageous sensitivities on contact with Fertisafe and Fertisafe Plus. In spite of the fact that these two disinfectants are VOC free and QAC free, professed to be embryo safe[14] still most extreme concerns exist with respect to their toxicity on embryos and gametes in ART set up.However, there is no independent study addressing this aspect.

Ethanol on other did not show 100% loss of Sperm motility in *direct contact*, sperms showed less sensitivity with ethanol relatively so ethanol can be used with good cautious around sperms.

Broadly, the experimental scenarios we created presents the example of worst case scenarios where *direct contact trial* proved to be Sperm lethal, extreme caution requires handling gametes therefore. For disinfection combination of disinfectants can be used.

As pH is a fundamental variable in an outer situation for gametes and growing embryos cultured*in vitro*. Occasional varieties to culture pH can be harming to regenerative cells as it can cause adverse impacts that prompts intracellular distress. [19] Since pH of culture medium influence sperm motility and Sperm binding [20] through Carbon dioxide and bicarbonates, hence any fluctuation in pH can have detrimental effects. In our study Fertisafe plus showed to fluctuate pH significantly toward acidic side and perhaps both Fertisafe and Fertisafe plus produced extreme Sperm sensitivity and motility loss due to acidic environment while remaining disinfectants did not show drastic variation in pH overall.

CONCLUSION

Our investigation revolved around disinfectants' potential toxicities in an attempt to find a universal disinfection protocol to aid ART setup on a conventionalplatform. We eventually found ethanol is safer relatively to be consumed around embryos and gametes. Possibly this finding is the strength of our study it strengthens further argument that we should not wait for poor case scenarios as in this study we constructed experimental conditions with extreme case scenarios. Coherence and standardisation of ART cleaning protocols a paramount important factor to prefer life expectancy of embryos and gametes while manipulation. Overall, with vigilance we can use a combination of different disinfectants. Steam sterilisation recently evolving disinfection technique in ART set up shown to be safer around gametes and embryos and aids on the development to blastocyst[21] although more research is required in this context,

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