

# A comparative analysis of grade 1 and grade 2 water in the Tygerberg Hospital *in vitro* fertilisation programme

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## Summary

There are conflicting reports in the literature about the effect of the water source for growth and insemination media in an *in vitro* fertilisation (IVF) programme. A controlled trial was carried out in which two-cell mouse embryos were cultured in Ham F10 medium containing either grade 1 water (test group) or grade 2 water (control group). Of the two-cell embryos, 92,0% (69 of 75) in the test group and 91,8% (67 of 73) in the control group cleaved to the blastocyst stage (no statistically significant difference).

On the basis of this experiment grade 2 water was used in the Tygerberg Hospital human IVF programme with good results. A continuing pregnancy rate of 23% per embryo transfer over a 1-year period is reported.

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The purity of the water used in preparation of the insemination and growth media, media composition and quality control are of the utmost importance in achieving success in an *in vitro* fertilisation (IVF) programme. Recently ultrapure high-pressure liquid chromatography grade water has been recommended for use in the culture of human embryos.<sup>1</sup>

In this study grade 1 and grade 2 water were compared using the mouse oocyte system. Grade 1 water (resistivity 18 mohm/cm) was obtained by single-stage reverse osmosis (Milli-R060 with a 5 µm carbon prefilter). A Rogers prefilter and a cellulose acetate R0-membrane were used. De-ionisation took place within a two-bed weak-base anion system (water obtained from Sabax Laboratories). Grade 2 water was also obtained from Sabax (AFF 7114 sterile Baxter water for irrigation).

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## Materials and methods

F1 hybrid female mice (C57 B1/6 x CBA) were treated with human menopausal gonadotrophin 10 IU followed by human chorionic gonadotrophin 10 IU 45 hours later to achieve super-ovulation. At the time of ovulation the females were mated with singly housed F1 hybrid studs. The females were sacrificed by cervical dislocation 44 hours after mating. Thereafter two-cell embryos were obtained.

Ham F10 media were prepared 24 hours before the experiment with grade 1 and grade 2 water. The medium containing grade 1 water was used in the test group and that containing grade 2 water in the control group. The osmolarity after preparation was 280 mOsm/kg (Wescor Inc. 5100 C).

The two-cell embryos were randomly divided into test and control groups. Both media contained 10% human serum and were exposed to 5% carbon dioxide-in-air for 24 hours before the experiment. The pH of the media, recorded with an 83 Autocal pH meter, ranged between 7,35 and 7,4.

The embryos were incubated for 72 hours (Forma Scientific 3157). The number of embryos in each group which reached the blastocyst stage was compared. Two experiments were performed over a 3-week period under identical conditions.

## Results

Sixty-nine of 75 embryos (92,0%) in the test group and 67 of 73 (91,8%) in the control group cleaved to the blastocyst stage (Table I). There is no statistically significant difference between the two figures.

TABLE I. CLEAVAGE OF TWO-CELL MOUSE EMBRYOS TO THE BLASTOCYST STAGE

Experiment	Test group (grade 1 water)		Control group (grade 2 water)	
	No.	%	No.	%
1	51/55	92,7	47/50	94,0
2	18/20	90,0	20/23	86,9
Total	69/75	92,0	67/73	91,8

## Discussion

Cleavage to the blastocyst stage was the same in the two groups. Grade 2 water is easily obtainable and commercially available in the RSA. It was decided to use this water in the human IVF programme launched in 1983 at Tygerberg Hospital. The first pregnancy followed soon after the programme was started<sup>2</sup> and led to the birth of the first IVF baby in the RSA on 29 April 1984.<sup>2</sup> Our fertilisation rate of 77,7% per oocyte has been satisfactory since May 1984<sup>3</sup> and compares favourably with that of leading IVF clinics,<sup>4</sup> as does the

pregnancy rate of 23% per transfer.<sup>5,6</sup> To establish our own water purification system to produce grade 1 water would mean a capital outlay of at least R10000. The average cost of obtaining grade 2 water is R1,40 per litre.

The basic classification allows for four water grades. The basis of this division is far from arbitrary and represents a specific range of water quality. The four basic grades must comply with the requirements listed in Table II.<sup>7</sup>

TABLE II. DIFFERENT GRADES OF WATER

Test	Grade 1	Grade 2	Grade 3	Grade 4
<b>Conductance</b> ( $\mu\text{S/cm}$ )	0,1-0,055	2-1	5-10	5-10
<b>Resistivity</b> (mohm/cm)	10-18	0,5-1	0,2-0,1	0,2-0,1
<b>Total dissolved solids (ppm)</b>	Limit of detection	< 0,05	< 0,05	< 0,05
<b>Organic solutes (ppm)</b>	Limit of detection	< 0,1	< 0,1	< 0,5
<b>pH range</b>	6,5-7,0	6,5-7,0	4-8,5	4-9,0
<b>Bacterial count (colonies/100 ml)</b>	Nil	Nil	< 50	As feed water
<b>Silica, SiO<sub>2</sub> (ppm)</b>	< 0,005	< 0,005	< 0,05	< 0,05
<b>Trace dissolved metals (ppm)</b>	< 0,005	< 0,005	0,005	As feed water

That water plays an important role in the success rate of an IVF programme has been well documented.<sup>8</sup> Whittingham<sup>9</sup> recommended the use of distilled water of the highest purity (resistivity of at least 0,7 mohm/cm) for culturing mouse embryos. As can be seen from Table II, this complies with

grade 2 water in terms of its quality attributes. Rain-water, glass-distilled six times, gave excellent results in human IVF work as well as in cleavage of mouse embryos in Adelaide.<sup>10</sup> Other workers use ultrapure high-pressure liquid chromatography grade water.<sup>1</sup>

There are conflicting reports in the literature on the best water to use in a human IVF programme. The use of grade 2 water gave good results in the Tygerberg Hospital human IVF programme, with a pregnancy rate of 23% per embryo transfer over a period of 1 year.<sup>3</sup> At times during that period a 38% pregnancy rate per embryo transfer was reached. The sterile Sabax Baxter water is easily obtainable, of high quality and low in price.

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## News and Comment/Nuus en Kommentaar

### Psycho-active drugs, doctors and medical students

The abuse of potentially addictive drugs by doctors has for many years been recognised as almost an occupational hazard because of the ease of access. In the USA, concern over those doctors whose abuse of alcohol and other drugs has impaired their capacity to practise has led to the establishment of local medical committees charged with assisting impaired doctors and their families towards rehabilitation.

From a survey conducted recently by the Harvard School of Public Health (McAuliffe *et al.*, *N Engl J Med* 1986; **315**: 805) it would seem that the problem is not too widespread. They surveyed 500 practising doctors and 504 medical students in a New England state and obtained a 70% and 79% response from the two groups respectively. It is not surprising to find that 59% of the doctors and 78% of the students had at some time in their lives taken a psycho-active drug for recreational use or for self-treatment. In the first category came mainly marijuana (dagga) and cocaine while self-treatment most often involved tranquillisers and opiates. However, most of this use was infrequent or simply experimental and only 10% of the doctors were currently using these drugs regularly while 3% had a history of drug dependence.

It has been found that in the USA about half the doctors who come to the notice of their colleagues as being addicted are addicted to alcohol while the other half are addicted to a wide range of other drugs. The present study did not inquire into alcoholic intake, so that it probably represents only about half the problem. Comparison of this study with earlier ones suggests that US doctors are not abusing drugs more than they did in past years and that the level of use in these medical samples should not be cause for great alarm. The recreational use of drugs by doctors and students simply reflects the current trends in drug use throughout American society. Doctors and students do not use amphetamines and such-like drugs 'instrumentally', i.e. to enhance capacity for study or otherwise to an extraordinary extent.

Nevertheless, as Lewis points out in accompanying editorial (*N Engl J Med* 1986; **315**: 826) the fact that the use of recreational drugs by young doctors has expanded beyond marijuana to the full spectrum of psycho-active drugs including cocaine, and that there has been a significant increase in cocaine use among medical students in recent years, gives cause for concern.

Prevention is more difficult than treatment, because it is not easy to define the population at risk or to avoid the stress and overwork that accompany a turning to use of drugs. Lewis believes that we must make a better job of identifying the population at risk and of making the social environment in which medical education and practice occurs a healthier one.