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Osmolarity studies with different containers and volumes in a human *in vitro* fertilization programme

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Summary

In performing *in vitro* fertilization, a stable osmolarity in the medium surrounding the egg or embryo is of the utmost importance if a good fertilization and pregnancy rate is to be achieved. This study evaluated osmolarity changes in different volumes of fluid in the Falcon 3001 and 3037 Petri dishes and the Falcon 2058 tissue culture tube over a 24-hour period. It was found that the osmolarity was more stable in the Falcon 3037 Petri dish and in the tissue culture tube. The 3037 Petri dish was chosen for culturing human embryos.

S Afr Med J 1985; **68:** 651-652.

Simulating physiological conditions in human *in vitro* fertilization work is of the utmost importance to achieve a good fertilization and pregnancy rate.[1] These factors are a pH of 7,4 in the growth medium, a stable temperature of 37°C, a high humidity of 98% and a constant osmolarity in the medium being used.

The osmolarity of the medium in different Petri dishes and in a tissue culture tube over a 24-hour period was evaluated.

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Method

Two tissue culture Petri dishes were chosen, the Falcon 3001 and the 3037, and a tissue culture tube, the Falcon 2058.

The osmolarity of the medium was always 282 mosm/kg after preparation. The same medium was used in all experiments. Different volumes of medium were used in the 3001 Petri dishes — 2, 4 and 5,5 ml; the 3037 dishes always contained 6 ml — 1 ml in the central well and 5 ml surrounding the well; and the 5 ml culture tube contained 1 ml. The containers were filled simultaneously with the different volumes and put into the incubator for 24 hours (Forma Scientific No. 3163).

Twenty-four hours later readings were taken with a 5100 C Vapor Pressure Osmometer, previously calibrated. A drop of medium was obtained from each holder in the incubator and the readings noted carefully.

This experiment was performed 9 times under similar conditions.

Results

The osmometer readings are shown in Table I. The original osmolarity at 0 hours was always 282 mosm/kg. After 24 hours the mean reading (± SD) in the Falcon 3001 with 2 ml fluid (group I) was 307 ± 4,11 mosm/kg, in the 4 ml volume dish (group II) it was 297 ± 3,71 mosm/kg and in the 5,5 ml (group III) it was 289 ± 2,42 mosm/kg. Mean readings in the Falcon 3037 (group IV) containing 6 ml of medium was 283 ± 2,08 mosm/kg and in the Falcon 2058 tube (group V) with 1 ml it was 284 ± 1,66 mosm/kg. The multiple-comparison procedure of Schaffe was used to compare the mean osmolarity levels after 24 hours at a significance level of $P = 0,05$.

Osmolarity levels in groups I, II and III differed from each other and from those in groups IV and V. Groups IV and V did not differ from each other in osmolarity levels but levels were significantly greater than the base value of 282 mosm/kg (t-test, $P < 0,05$).

The stability of the osmolarity in the five containers varied from group I as the least stable to group V as the most stable. This can be seen by looking at the coefficient of variance in Table I.

TABLE I. OSMOLARITY READINGS — 24 HOURS (282 mosm/kg AT 0 h)

Experiment	Group I (3001) 2 ml	Group II (3001) 4 ml	Group III (3001) 5,5 ml	Group IV (3037) 6 ml	Group V (2058) 1 ml
1	309	301	287	282	283
2	301	297	286	282	282
3	302	296	288	283	286
4	310	302	289	287	287
5	309	302	293	287	285
6	313	291	288	283	283
7	309	298	289	282	283
8	308	295	289	285	284
9	303	295	293	284	283
Mean	**307,11**	**297,44**	**289,11**	**283,88**	**284,0**
SD	**4,11**	**3,71**	**2,42**	**2,08**	**1,66**
CV	**1,337**	**1,248**	**0,837**	**0,714**	**0,584**

SD = standard deviation; CV = coefficient of variation.

Discussion

Osmolarity studies on follicular fluid and human serum by Edwards[2] have led to the use of a medium osmolarity of 280 - 285 mosm/kg by various workers.[3,4] A stable osmolarity of the medium surrounding the oocyte or embryo is of great importance in human *in vitro* fertilization work.[1] Two factors play a role in achieving a stable osmolarity in a specific container — the surface area and the volume. In Petri dishes with a large surface area a change in osmolarity is obvious if the volume is small; however, the readings are much more stable when the volume is increased (Table I). The volume is not as important when a tissue culture tube is used because the surface area is small.

After our study on osmolarity the Falcon 3037 Petri dish was chosen for use because the osmolarity is fairly constant, and because if tubes are used for culturing the oocyte there is a greater chance of losing the egg or embryo.[3] The Falcon 3037 Petri dish had also been used for some time with success and good pregnancy results by the Norfolk group.[4,5]

Osmolarity studies were also performed on the multi-dish system used in Europe by different workers.[3,6] Lauritzen *et al.*[6] noticed an increase in osmolarity when an open-dish system was used because of evaporation. The use of only one incubator with frequent opening and closing of the door can lead to a change in osmolarity due to loss in humidity and evaporation of the medium.

The use of paraffin oil was abandoned by the Royal Womens' Hospital group in Melbourne because of its potential toxicity. An increase in volume of culture medium and better control of humidity within the incubator was necessary to achieve a stable atmosphere. An additional incubator was thus installed to create better culture conditions.[7]

After implementation of this basic knowledge as part of the quality control in our human *in vitro* fertilization programme, the first pregnancies soon followed.[8]

The authors wish to thank Mrs. H. Krüger for the preparation of this manuscript, Sister H. Rosich, our research assistant, and the South African Medical Research Council for help in the statistical analysis.

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