RENEWAL OF CULTURE MEDIA ON DAY 4 OF HUMAN IVF EMBRYO DEVELOPMENT RESULTS IN A HIGHER BLASTULATION RATE WHEN COMPARED TO SUPPLEMENTATION OF THE MEDIA

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ABSTRACT

Introduction: Elective single embryo transfer is becoming widely implemented in IVF and is dependent on the selection of an embryo with the highest developmental potential. Blastocyst transfer may assist in this selection but requires optimization of culture techniques. During blastocyst culture, changes of media are advocated to deplete embryo-toxic substances and reduce naturally occurring inhibitors. However, this can cause embryonic stress. Furthermore, embryonic genome activation leads to a rise in the production of embryo proteins, growth factors and receptors which are developmentally beneficial[1,2] but are removed upon transfer to new media. This prospective controlled study investigates the effects of continuing culture, after day 4, in the same media.

Methods: From a pilot study, 266 embryos were determined necessary to complete this study. All supernumerary day 3 embryos from consenting patients were included if they had a minimum of 5 cells and less than 50% fragmentation. Day 3 embryos were randomly allocated to each of the two groups. Up to day 4, all embryos were cultured individually according to our standard protocol. In the media renewal group, each embryo was transferred to a freshly prepared 40µl equilibrated media drop on the afternoon of day 4. Also on the afternoon of day 4, in the media supplementation group, 20µl of warmed equilibrated culture media was added to the 20ul drop in which the embryo was being cultured. Embryos were scored on days 5 and 6 and the quality of each blastocyst recorded.

Results: From the 134 embryos in the supplementation group, 30 blastocysts were observed on day 5 and 54 blastocysts on day 6 giving blastulation rates of 22.4% and 40.3% respectively. From the 94 embryos in the renewal group, there were 36 blastocysts on day 5 and 56 blastocysts on day 6 giving blastulation rates 38.3% and 59.6% respectively. The number of blastocysts was statistically significantly higher in the renewal group both on day 5 (p= 0.009) and on day 6 (p=0.005).

Conclusions: We demonstrated that, under our culture conditions, a greater number of blastocysts were observed if the culture media was renewed on day 4 of development rather than supplementing the media. Based on these findings, blastocyst culture in our laboratory involves transfer of all embryos into freshly prepared media on day 4.

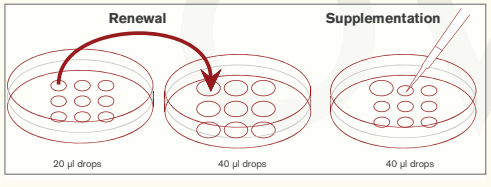
OBJECTIVE

The aim of the present study was to evaluate the blastulation rate after embryos were cultured in one of two extended culture systems. On day 4, the embryos were either transferred into fresh media or media was supplemented to the embryo current culture drop.

METHODS

A controlled prospective study was performed between March and September 2010 at OVO FERTILITY in Montreal. A total of 97 couples were included in the study, providing a range of 1 to 11 embryos, with a median of 2 embryos donated per couple. The supernumerary embryos from consenting patients were included in the study if they developed to at least 5 cells with no more than 50% fragmentation on day 3. Donated embryos included those with abnormal scoring at fertilization check (1 and 3PN or 0PN2PB), suboptimal pronuclei scores (Z4), abnormal development such as multinucleation, and poor embryo morphology.

Embryos were cultured with COOK sequential media (COOK Canada). Each embryo was transferred from cleavage media to blastocyst media on the afternoon of day 2. Single embryo culture was performed in 20µl droplets under oil (COOK). After clinical transfer, all eligible embryos were randomly assigned to one of the two culture systems. On the afternoon of the 4th day of culture, embryos allocated to the renewal group were transferred to a new 40µl equilibrated media drop (COOK blastocyst), whereas 20µl of fresh equilibrated media (COOK blastocyst) was added to the embryos in the supplementation group. All embryos were cultured under reduced oxygen in G-185 tri-gas incubators (K-system). On the 5th and 6th day of culture, the embryos were observed and blastocyst formation was recorded. The differentiation of cells into a trophectoderm and an inner cell mass with a visible blastocoel was scored as a blastocyst independent of the quality. Blastocyst formation or developmental arrest was measured as a binary result. Blastocyst quality was also recorded for further analysis. Some research embryos were excluded from the study due to non-compliance with the inclusion criteria and/or study protocol.



STATISTICS

Statistical analysis was performed using a two tailed Fisher exact test with 80% power and significance at 0.05. Based on a pilot study, the sample size required was calculated to be 121 embryos in each group. To incorporate a dropout rate of 10% the sample size required was 266 embryos. A randomized number generator program (for integers 1 and 2 where 1 was supplementation and 2 renewal groups) was used to randomize the embryo culture techniques.

RESULTS

	Media Renewal	Media Supplementation	p-value
Number of embryos	94	134	
Day 5 Number of blastocysts Blastulation rate (%)	36 38.3	30 22.4	0.009
Day 6 Number of blastocysts Blastulation rate (%)	56 59.6	54 40.3	0.005

Of the 266 embryos initially recruited, 16 and 22 were excluded from the renewal and supplementation groups respectively. The blastulation rate for each type of embryo included in the study ranged from 34% to 71% with the lowest rate generated from multinucleated embryos and the highest from 0PN2PB. However, the distribution of each type of embryo into each culture system was similar.

DISCUSSION

This controlled study demonstrates that transferring embryos into fresh media on the 4th day of culture leads to a statistically significantly higher rate of blastocyst formation compared to supplementing fresh media to the drop where the embryo was already cultured for 48 hours. The difference in blastulation rate observed in the two culture systems could be explained by the rise of ammonium in the media due to amino acid breakdown. It's been observed that reducing ammonium toxicity improves embryo viability and developmental competence[3]. Periodic changes of media avoid increase in the levels of ammonium. Contrastingly, transferring embryos from one culture media to another could cause osmotic, metabolic, oxidative, pH and thermal stress to the embryo. In addition, it has been suggested that the presence of specific factors secreted by the embryo could optimize blastocyst development[4,5], favoring the supplementation system. However this was not supported by the results of our study.

One possible limitation of our study is the utilization of suboptimal embryos. It could be that the interactions of these embryos with their environment differs from that observed with optimal embryos. However, it is possible to generate viable pregnancies from many of the types of embryos included in the study, although at a lower rate than from optimal embryos, and therefore, we believe the results from this study are useful in clinical practice.

Furthermore, we observed that embryos in the renewal group had a tendency of being of better quality than embryos in the supplementation group. Fifty percent of the blastocysts formed were of good quality in the renewal system (2BB or better quality[6]) versus 40% of blastocysts in the supplementation system. However no statistical analysis was performed because of the small size of the groups.

From a clinical perspective, this improved blastocyst culture technique could optimize blastulation rate and embryo viability and as a result increase the proportion of embryo transfers at the blastocyst stage which will increase the implantation rate and therefore reduce the transfer of multiple embryos and resultant multiple pregnancy rate.

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