ABSTRACT

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 superovum 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 (2+4), 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 20 µl 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 20 µl equilibrated culture medium, whereas 20 µl of fresh equilibrated media (COOK blastocyst) was added to the embryos in the supplementation group. All embryos were cultured at 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-conformity with the inclusion criteria and/or study protocol.

RESULTS

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 because of the small size of the groups. However, 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 has been observed that reducing ammonium toxicity improves embryo viability and developmental competence. 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, thus favoring the supplementation system. However, this was not supported by the results of our study.

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