A single medium and sequential media are equally effective for the culture of human embryos to the blastocyst stage: a pilot study

by Danielle T Schneider, Sidney Verza Jr. & Sandro C Esteves

ANDROFERT, Centro de Referência para Reprodução Masculina, Campinas, São Paulo, Brazil

To be presented, American Society for Reproductive Medicine 2009 Annual Meeting, Atlanta, GA, P-510.

INTRODUCTION

Several culture media have been designed for blastocyst culture based on the composition of oviduct and uterine fluids and on the patterns of embryo metabolism. Two kinds of culture media are used to culture the embryos until day 5: [1] a single medium is used throughout culture to the blastocyst stage) and [2] two media of different composition are used sequentially.

In sequential media culture systems, the medium used for culture from Days 1 to 3 of development differs in composition and/or concentration of the components from the medium used for subsequent culture from day 3 to the blastocyst stage. In a single medium system, the composition and the concentration of the components are the same from Day 1 to Day 5 (1).

In general, there are many arguments in favor of sequential media: the changing energy requirements of the preimplantation embryo, with the inhibitory effect of glucose on early cleavage stage embryos and the inhibitory effect of ethylenediaminetetraacetic acid (EDTA) on blastocyst development and the inner cell mass (ICM); the chemical breakdown of L-glutamine (Gln) in aqueous solution, resulting in accumulation of ammonia, that affects embryo development; and the role of amino acids on preimplantation embryo development (2). On the other hand, others have shown similar results for blastocyst culture using either the single medium or sequential media systems (1-3).

The choice of the best culture medium is an important challenge for the embryologist, and should be based on both (i) published studies and (ii) in-house studies in each IVF laboratory, in order to determine whether the published findings are applicable to the specific work conditions.

OBJECTIVE

The aim of this study was to compare in-vitro development for extended culture until Day 5 for embryos cultured in a single medium (Global), with embryos cultured in sequential media (G-1/ G-2).

MATERIALS and METHODS

A total of 477 cumulus-oocyte complexes retrieved for intracytoplasmic sperm injection (ICSI) were blindly randomized for culture. After ICSI, the injected oocytes were cultured in two different media systems, as follows, Group 1: Global (LifeGlobal, IVFonline, Canada n=231, Day 0 to Day 5) and Group 2: G-1 (Vitrolife, Sweden, Day 0 to Day 3, n=175) and G-2 (Day 3 to Day 5).

Ovulation Induction and Oocyte Recovery

Ovarian hyperstimulation was achieved using GnRH analogue (nafarelin acetate, Synarel®, Pfizer) along with human recombinant FSH (r-hFSH: Gonal-F®, Merck-Serono) in a long down-regulation protocol. Starting gonadotrophin dose varied from 150-300 IU and was based on female age, body mass index, ovarian volume, basal FSH levels and the number of preantral follicles. Human chorionic gonadotrophin (hCG; Ovidrel®, MerckSerono) was administered when at least one follicle reached 18 mm in diameter on ultrasound scan. Oocytes were collected 34-36 hours after hCG administration under transvaginal ultrasound guidance.

Preparation of Oocytes and Spermatozoa

Cumulus-oocyte complexes were treated with 80 IU/ml hyaluronidase for 30 seconds (Hyase, Vitrolife, Sweden) and then stripped of the remaining cumulus cells by mechanical aspiration through a 130-μm pipette (Flexipet, Cook, USA). Ejaculated sperm samples were processed for ICSI by using discontinuous two-layer density gradient or simple washing. Percutaneous
epididymal (PESA) or testicular (TESA) sperm aspirations were performed to retrieve sperm from men with obstructive azoospermia. Testicular sperm aspirations (TESA) or testicular sperm extraction with microdissection (micro-TESE) were used in non-obstructive azoospermia cases (4).

**Intracytoplasmic Sperm Injection (ICSI) and Embryo Culture**

Oocytes showing first polar body extrusion were injected with a single spermatozoon. Sperm selection and microinjection were performed using 400X magnification. The microscope and the manipulators were setup on anti-vibratory table. All injections were performed at 37°C on an inverted microscope (Eclipse, Nikon, Japan) equipped with Hoffman modulation contrast optics, and electro-hydraulic manipulators and microinjectors (Narishige, Japan). Injected oocytes were washed and transferred to 50 μL droplets of either Global (Group 1) or G1 (Group 2). Fertilization was checked 18-20 hours after ICSI. Normally fertilized zygotes were then transferred to microdroplets of either Global (Group 1) or G1 (Group 2) covered with mineral oil (Ovoil, Vitrolife, Sweden) at 37°C in a humidified atmosphere of 6.0% CO2 in air. In both groups, embryos were transferred to a new drop everyday and the culture dish was changed every 2 days. Embryos in Group 2 were transferred to microdroplets of G2 on Day 3. Micromanipulation of the gametes and embryo culture were carried out in an ISO 5 clean room equipped with volatile organic compounds (VOC) filtration units (VECO, Brazil) (5).

**Main Outcomes Measures**

Fertilization rate and the proportion of top quality embryos (TQE) on days 2 and 3 of culture were compared. TQE were defined as those presenting with 3-4 and 7-9 equal-sized blastomeres on days 2 and 3 respectively, with grades I or II of cytoplasmic fragmentation, ≥1 blastomeres with visualized nucleus, both pronuclei (PN) with ≥4 nucleoli and with the same size. The proportion of zygotes that reached the morula stage on day 4 and blastocyst on day 5 was also compared between groups. Blastocyst classification were performed and compared between groups. The blastocysts were classified as fully expanded (Figure 1a), non-expanded (Figure 1b) or early (Figure 1c) (6). The fully expanded blastocyst is defined as having an almost non-existent zona, single-layered trophoblastic cells, and an inner cell mass which is out of focus, but identifiable. The non-expanded blastocyst has relatively large cells and delineation between the trophoblastic and inner mass cells, while the early blastocyst has a thinning zona and accumulation of cells to one pole.

**Statistical Analyses**

Student’s t-tests and Mann-Whitney U tests were used to compare the groups, as appropriate, using commercially available software. P<0.05 was considered significant.

**RESULTS**

The number of injected mature oocytes was significantly higher in Group 1 as compared to group 2, due to the fact the proportion of mature oocytes was significantly higher in the former. It should be noted, however, that randomization was performed immediately after oocyte pick-up, before the assessment of oocyte maturation. There were no significant differences in fertilization and blastocyst formation rates between groups (Table 1). There was a trend towards better quality embryos on days 2 and 3 in favor of the single medium (Group 1), and a significantly higher morula formation rate in this group. Blastocyst quality did not differ between groups.

**Figure 1:** Morphological classification of blastocysts.
DISCUSSION and CONCLUSION

The similar blastulation rate in both single and sequential culture systems shows that the single medium is as effective as the sequential media for the development of human embryo blastocysts. However, the single medium system offers some characteristics for the embryologist that make the laboratorial management easier than sequential media, because (i) only one medium is used for all steps of embryo development, (ii) there is no risk of culture medium interchange during preparation of culture dishes, and (iii) higher frequency of bottles opening and consequently fresh culture medium available to prepare the dishes.

References


You can contact Sandro C. Esteves, MD, PhD, Director at: s.esteves@androfert.com.br

Table 1: Comparison of fertilization and embryo formation up to the blastocyst stage of injected human oocytes cultured either in a single medium (Group 1; Global) or in sequential media (Group 2; G-1/G-2). Values are mean ± SD.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (Global)</th>
<th>Group 2 (G-1/G-2)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injected oocytes (n)</td>
<td>7.9±2.9</td>
<td>6.0±3.3</td>
<td>0.004</td>
</tr>
<tr>
<td>2PN fertilization rate (%)</td>
<td>69.5±21.3</td>
<td>62.6±17.6</td>
<td>0.189</td>
</tr>
<tr>
<td>Top quality embryos on Day 2 ¹ (%)</td>
<td>72.1±18.5</td>
<td>56.1±33.2</td>
<td>0.067</td>
</tr>
<tr>
<td>Top quality embryos on Day 3 ¹ (%)</td>
<td>50.3±28.3</td>
<td>35.2±33.8</td>
<td>0.055</td>
</tr>
<tr>
<td>Morula formation rate on Day 4 (%)</td>
<td>20.8±25.2</td>
<td>7.5±17.6</td>
<td>0.023</td>
</tr>
<tr>
<td>Blastulation rate (%)²</td>
<td>40.8±22.9</td>
<td>42.2±37.6</td>
<td>0.975</td>
</tr>
<tr>
<td>Fully expanded blastocyst rate (%)³</td>
<td>13.4±17.3</td>
<td>10.6±17.1</td>
<td>0.549</td>
</tr>
<tr>
<td>Non-expanded blastocyst rate (%)⁴</td>
<td>14.6±16.2</td>
<td>16.9±27.5</td>
<td>0.691</td>
</tr>
<tr>
<td>Early blastocyst rate (%)⁵</td>
<td>12.9±16.8</td>
<td>14.6±25.5</td>
<td>0.749</td>
</tr>
</tbody>
</table>

¹ Embryos with 3-4 and 7-9 blastomeres on day 2 and 3, respectively, with grade I or II of fragmentation.
² Proportion of zygotes that reached the blastocyst stage on Day 5 of culture.
³ Blastocyst with an almost non-existent zona, single-layered trophoblastic cells, and an inner cell mass which is out of focus, but identifiable.
⁴ Blastocyst with relatively large cells and delineation of trophoblastic and inner cells lines.
⁵ Blastocysts with thinning zona and accumulation of cells to one pole.