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Comparison of Nine Media in the Culture of Human Ovarian Granulosa Lutein Cells

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Abstract. *Background: Cultures of human ovarian granulosa lutein (hGL) cells are broadly used in experimental studies. The choice of the culture medium is important for the optimization of the conditions for culture of hGL cells. Aim: To compare the efficiency of a basic salt solution and eight different defined media on the culture of hGL cells. Materials and Methods: Cultures of the HGL-5 cell line were maintained for 72 hours with DMEM/F12, RPMI-1640, Ham'sF10, Modified Ham'sF10®, HTFXtra®, Global®, Complete Multiblast®, Universal® or Earle's balanced salt solution (EBSS). At the end of the culturing period, the attachment, the viability and the total number of cells were measured. Results: Culture in DMEM/F12 led to the highest score of all studied parameters, followed by RPMI-1640. The lowest performance was recorded with Complete Multiblast® and EBSS. The use of the other media gave mediocre results. Conclusion: Among the media tested, DMEM/F12 appears to be the best choice for the culture of hGL cells.*

Human ovarian granulosa cells are of critical importance during oocyte development. They secrete a plethora of factors depending on the phase of development, of which the most important are estradiol (E-2) and progesterone (1).

During the first half of menstrual cycle, the prominent product of ovarian granulosa cells is E-2. Granulosa cells express follicle-stimulating hormone (FSH) receptors and under the influence of FSH express aromatase that converts androgens, produced in theca cells, to E-2. During the luteinizing hormone (LH) surge, granulosa cells turn into granulosa lutein (hGL) cells and their secretion profile changes; they start producing mainly progesterone and the production of E-2 is reduced although not ceased (1).

hGL cells are used in *in vitro* experiments mainly for two reasons. Firstly, their function, namely their secretory profile, is of paramount importance for final oocyte maturation and the efficient function of *corpus lutea*. Secondly, it is easy to obtain hGL cells during follicular aspiration that takes place in *in vitro* fertilization (IVF) cycles. However, hGL cells are limited in number and have a limited life span *in vitro* (2).

These problems were solved with the generation of immortalized hGL cell lines, capable of continuous propagation (3). HGL-5 is an immortalized cell-line derived from primary hGL cells after transformation with the E6 and E7 regions of human papillovirus 16 (4). HGL-5 cells are able to grow quickly and to form large cultures; they have the ability to produce progesterone and to convert androstenedione to E-2 and in general, they retain many of the functions of primary hGL cells; therefore they are a useful model for the investigation of hGL function (3, 4).

Culture medium is essential in cell cultures, providing cells with necessary substances and at the same time maintaining pH and osmolarity within the appropriate range in order for cells to grow. In general, culture media are distinguished into balanced salt solutions and defined media. A balanced salt solution is a simple medium containing inorganic salts and usually glucose. Defined media are complex media composed of inorganic salts, essential and often non-essential amino acids, energy sources, vitamins, organic supplements and sometimes growth factors and hormones. The complexity of defined media varies, therefore their efficiency for the growth of certain cell lines also varies (5).

In this study, we compared the efficiency of several media for the culture of HGL-5 cells. We tested common media especially designed for cell culture, such as Dulbecco's modified Eagle's medium/F12 (DMEM/F12),

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Table I. *Percentage of attached HGL-5 cells, total number and percentage of live HGL-5 cells at the end of the 72-hour culture period. The mean±standard error is given, as well as the minimum and maximum values in parentheses (n=30). Values with the same superscript letters were not significantly different. In all other cases, values were significantly different (p<0.05).*

Group	Culture medium	Supplier	Attachment (%)	Total no. of cells (×1000)	Vitality (%)
1	RPMI-1640	Invitrogen, GIBCO, Karlsruhe, Germany	92.27±0.30 (90-95)	798.33±8.919 (700-860)	86.25±0.35 (82-89)
2	Ham F10 Heppes	Sigma-Aldrich, Saint Louis, Missouri, USA	57.43±0.327 (55-60)	522.66±9.492 (450-610)	71.22±0.322[b] (68.7-74)
3	HTF Xtra®	Life Global, Canada	63.2±0.312 (60-65)	575±9.693 (500-650)	73.43±0.194[b] (71.8-75)
4	Global®	Life Global, Canada	66.7±0.287 (65-70)	624.33±7.606[a] (570-730)	73.9±0.156[b] (73-76)
5	Modified Ham's F10®	Irvine Scientific, Santa Ana, California, USA	72.6±0.329 (70-75)	623.66±9.971[a] (530-720)	76.8±0.224 (75-79)
6	DMEM/F12	Invitrogen, GIBCO, Karlsruhe, Germany	95.3±0.349 (92-98)	882±9.14 (800-980)	93±0.358 (88.7-96)
7	MultiBlast®	Irvine Scientific, Santa Ana, California, USA	43±0.383 (40-45)	386±9.5 (310-480)	58.5±0.228 (56.8-60.5)
8	EBSS	Invitrogen, GIBCO, Karlsruhe, Germany	52.6±0.337 (50-55)	489.66±7.103 (420-550)	63.7±0.191 (62-65.7)
9	Universal IVF®	Origio, Jyllinge, Denmark	67.6±0.326 (65-70)	604±10.727 (520-690)	73.6±0.186[b] (72-75.2)

Roswell Park Memorial Institute medium (RPMI)-1640 and Ham's F10, as well as a series of media especially designed for the culture of human gametes, embryos and ovarian cells such as Modified Ham's F10®, Global®, HTFXtra®, Universal IVF® and Complete Multiblast medium®. We also tested the efficiency of Earle's balanced salt solution (EBSS). The efficiency of the culture media was evaluated regarding three outcome measurements: the ability to attach to the substrate, the proliferation and the viability of HGL-5 cells.

Materials and Methods

HGL-5 cells were used in all cultures. The cell line was generously provided by the Department of Gynecology and Obstetrics, University Hospital of Schleswig-Holstein, Campus Lübeck, Germany. A total of 270 cultures were established in 6-well plates. In each culture, 100,000 live cells were seeded into a well and cultured for a 72-hour period with 2 ml of culture medium. Depending on the culture medium the cultures were divided into nine groups as shown in Table I.

Each group included 30 cultures. The culture plates were incubated in a Heracell 150 incubator (Thermo Electron Corporation, Langenselbold, Germany), at 37˚C, in an atmosphere of 5% CO_2 and high humidity. After 72 hours, the wells were inspected under a dissecting microscope in order to evaluate the percentage of attached cells. The evaluation of the percentage of attached cells was performed by the same person in all cases. Afterwards, the cells were detached with the use of Accutase (Innovative Cell Technologies Inc., San Diego, CA, USA), washed in PBS (Biochrom AG, Berlin, Germany) and the vitality was evaluated with the Trypan blue exclusion test (Sigma-Aldrich, Singapore). The precise measurement of the total number of all, as well as the number of live HGL-5 cells, was performed with an improved Neubauer haemocytometer.

Statistical analysis was performed with Statistica 6.0 (StatSoft Inc., Tulsa, OK, USA). This included descriptive statistics and comparisons between the experimental groups with Kruskal ANOVA and Mann-Whitney *U*-test regarding the following parameters: total number of cells, percentage of live cells (vitality) and percentage of attached cells. Differences were considered significant at values of $p<0.05$.

Results

The seeded HGL-5 cells attached to the wells within the first 12 hours of culture. Group 6 (DMEM/F12) had the highest percentage of attached cells, followed by group 1 (RPMI-1640). In group 7 (Multiblast®), less than half of the HGL-5 cells attached to the wells. After 72 hours' culture, HGL-5 cells proliferated in all groups and formed dense cultures, especially when incubated with DMEM/F12 and RPMI-1640. Group 6 (DMEM/F12) had the highest number of cells, followed by group 1 (RPMI-1640). In contrast, group 7 (MultiBlast®) and group 8 (EBSS) resulted in the lowest numbers of cells. The remaining culture media led to mediocre percentages of adhesion and proliferation. The same ranking was also found for the percentage of live cells (Table I).

Discussion

According to the results, DMEM/F12 proved to be the most suitable medium for HGL-5 cell culture. It seems that its composition fully meets the nutritive requirements of these

cells. RPMI-1640 also gave excellent results, although inferior to those of DMEM/F12. DMEM/F12 is particularly rich in salts, vitamins, amino acids and energy sources (6). RPMI-1640 is a medium traditionally used for human lymphoid cells. It has a similar composition to DMEM/F12, without sodium pyruvate and L-alanine, having a pH of 8 (7).

The poor results with EBSS were expected, as this is a simple salt solution without critical factors in its composition, such as amino acids, vitamins and adequate sources of energy (8).

The inclusion of the other media in this study was based on the fact they contain salts, vitamins and energy sources which are able to support the culture of gametes, embryos and ovarian cells (9), therefore they should support the growth of hGL cells as well. In particular, Ham's F10 was originally designed to support the growth of Chinese Hamster ovary and human cell lines (10). Modified Ham's F10® is a formulation based on the classical Ham's F10 without hypoxanthine. HTFXtra® is a medium based on the composition of human oviductal fluid (11), enriched with selected non-essential amino acids. Global® comes from the development of KCl simplex optimization medium (KSOM) (12). Universal IVF® is an Earle's-based, high glucose medium with synthetic serum replacement (13). However, the results of the present study indicate that these media are not suitable for hGL cell cultures. Complete Multiblast® Medium led to the worst results. This is a medium containing five inorganic salts; three energy substrates; sodium citrate as antioxidant; alanyl-glutamine; thirteen essential and seven non-essential amino acids; and dextran serum supplement. Although its composition is more advanced than that of EBSS, HGL-5 cell cultures with Complete Multiblast® had a poorer outcome than those with EBSS.

Given that the composition of most of the tested culture media is not fully known, we are not able to provide in-depth explanations for the results. However, the present study clearly showed that among the tested media, DMEM/F12 is the most suitable culture medium for hGL cells. RPMI-1640 was also shown to be a good alternative culture medium.

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