Oocyte secreted factors receptors in a granulosa cell line.

An approach to oocyte quality assessment

Reis SA[1], Ribeiro A[1,2], Silva-Carvalho JL[1,2,3], Gouveia AM[1,4], Almeida H[1,3].

1- Departamento de Biologia Experimental, Faculdade de Medicina, IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto;
2 - CETI – Centro de Estudo e Tratamento da Infertilidade, Porto.
3 – Hospital CUF., Porto.
4- Faculdade de Ciências da Nutrição e Alimentação, Universidade Do Porto.

Introduction

The granulosa cells (GCs) have a role in the oocyte development and follicle growth and, therefore, are contributors to oocyte quality. GCs differentiate in mural GCs, lining the follicle, and cumulus oophurus cells (CCs), that surround the oocyte in a close and tight relationship. The oocyte synthesizes and releases oocyte-secreted factors (OSFs) such as GDF9 and BMP15, measurable in the follicular fluid. This OSFs mediate the activation of SMADs and MAPK pathways through the activation of TGF β family receptors in CCs. However the mechanisms involved are not yet completely understood. As primary cell cultures are difficult to establish and cell lines behave in a more predictable way, we reasoned that the use of a GC cell line would increase our knowledge about folliculogenesis.

Due to the similarity of GC1a cell line with CCs, this cell line was employed as a tool to study the interaction between oocytes and CCs. However this cell line was never characterized, it is our aim to approach that in order to increase our understanding about the mechanism involved in oocyte quality.

Aims

With this work we pretend:
- To characterize the presence of OSFs and their receptors in GC1a cell line;
- To analyze the influence of follicular fluid in growth and morphology of GC1a cells in culture.

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Reference

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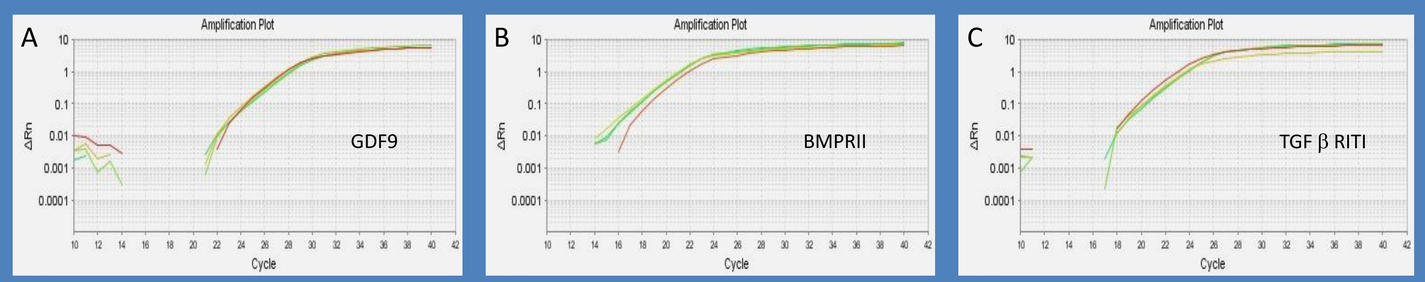
Results

A. Evaluation of OSFs and their receptors transcripts in the GC1a cells



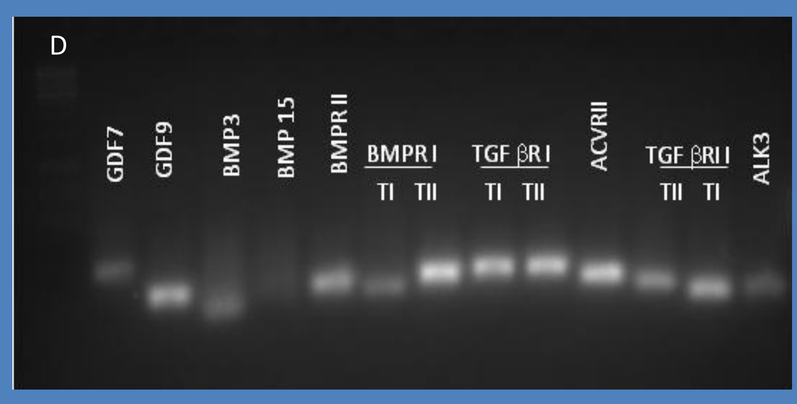


Fig . 1- Detection of OSFs receptors transcripts in GC1a cells. The GC1a cell line was cultivated in DMEM with 10% FBS. The total mRNA was extracted using Ambion kit, converted in cDNA employing 5 μg of mRNA. To analyze gene expression, real-time PCR was performed using SYBR Green. Upper panel - Amplification curve of GDF 9 (A), BMPRII (B), and TGF β RITI (C). D - Each RT- PCR product was direct confirmed by agarose gel (2% of agarose); Confirmed the expression of OSFs (GDF7, GDF9, BMP15) and their receptors (BMPR I, BMPR II, TGF β RI, TGF β RII, ACVR II, ALK 3).

B. Morphology of GC1a cells treated with follicular fluid

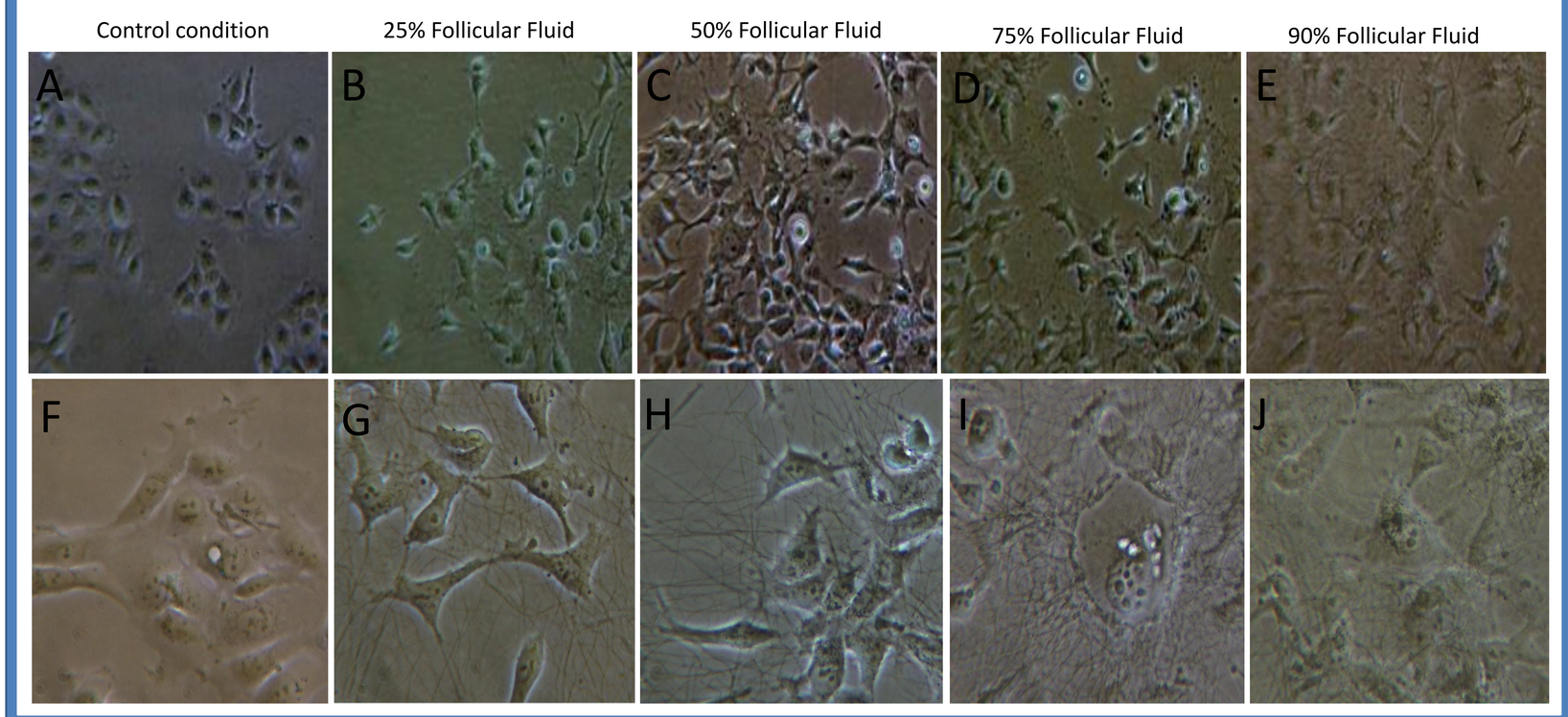


Fig . 2- Microscopic aspect of GC1a cell line. Upper panel - A - GC1a cell line was cultured in DMEM with 10% FBS (control); B,C,D and E - GC1a cell line maintained in DMEM with 25%, 50%, 75% and 90% of follicular fluid during 24h, respectively. x 100. Lower panel - F - GC1a cell line was cultured in DMEM with 10% FBS (control); G,H,I and J - GC1a cell line maintained in DMEM with 25%, 50%, 75% and 90% of follicular fluid during 24h, respectively. x 200.

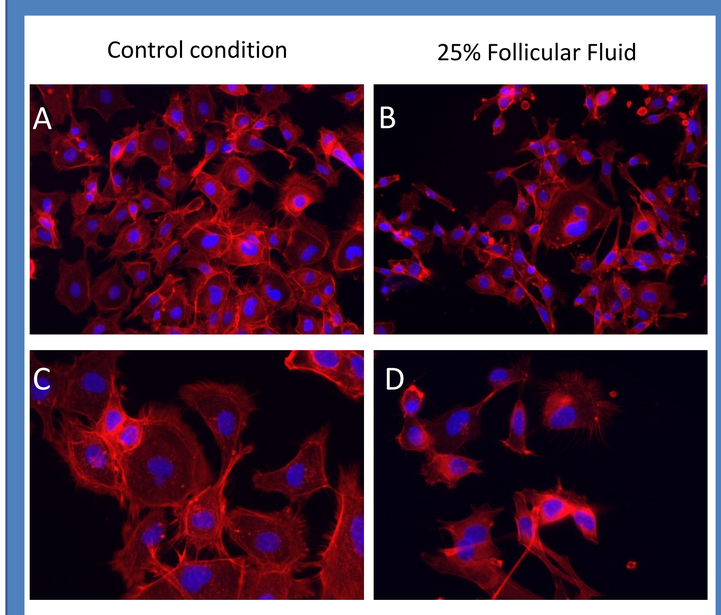


Fig . 3- Actin cytoskeleton organization in GC1a cell culture with 25% follicular fluid. Left panel- GC1a cell line was cultured in DMEM with 10% FBS (control), actin were staining with phalloidin and the nucleus were staining with DAPI; Right panel - GC1a cell line was cultured in DMEM with 25% Follicular Fluid , actin were staining with phalloidin and the nucleus were staining with DAPI;
A- GC1a cells with epithelial morphology (200x), more detail in C (400x); B - GC1a cell with alteration in the morphology more close than fibroblastic morphology (200x), more detail in D (400x).

Conclusion & Discussion

To the best of our knowledge this is the first work that evidences OSFs and OSFs receptors expression in GC1a cells. Follicular fluid concentration appears to influence cell morphology suggesting that controlled conditions as specific culture medium and added specific growth factors ought to be employed. In cultures supplemented with follicular fluid the cells lose their epithelial like morphology and have a more closed fibroblastic morphology, with cellular protrusions. This data is supported by the analyze of actin staining, but need to be complemented with the study of cytoskeleton reorganization.
As a whole, the study establishes a consistent ground for future work in the TGF β transductive pathways. This includes the modulation by OSFs and the following activation of intracellular second messengers.

GC1a cells in culture mimic CCs in expression of TGF β family receptors, supporting their use in the study of downstream pathways involved in oocyte maturation.