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Abstract Title
C1q as pro-angiogenic factor in the context of ovarian tissue transplantation

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Abstract Body
Problem
Anti-cancer therapy is increasingly effective, nonetheless, in women, it can result in iatrogenic premature ovarian insufficiency. In fact, follicle depletion is triggered by chemotherapy which induces apoptosis, interferes with cellular pathways responsible for quiescence maintenance, and damages the vascular network. For pre-pubertal girls and young women needing immediate therapy, the only option for fertility preservation is ovarian tissue cryopreservation and transplantation. A large part of follicle reserve is lost as a consequence of hypoxia due to an incomplete graft revascularization [1,2]. Ovarian tissue transplantation supplemented with autologous endothelial cells from ovary could result in a successful engraftment. Since previous work [3] already demonstrated that C1q could have a pro-angiogenic role, we decided to use this complement factor to enhance revascularization in this context.

Method of Study
OVarian Endothelial Cells (OVECs) were isolated from ovarian biopsies from patients undergoing ovariectomy/annessiectomy. Tissue was digested by trypsin and, subsequently, by collagenase type I. Purification was achieved with a mixture of Dynabeads® CD31 and UEA-lectin (Ulex Europeus Aglutinine-Lectin)-conjugated beads. Angiogenic assays were performed in presence of C1q. The migration assay was conducted in a transwell system seeding cells in the upper chamber; the lower chamber was loaded with C1q and the percentage of migrated cells was calculated. In wound healing assay the monolayer of cells was stripped and C1q was added; the percentage of wound closure was estimated. For the tube formation assay cells were seeded in Matrigel® in presence of C1q; the following day, tubes were counted. We performed RT-qPCR to evaluate the expression of genes involved in the angiogenic process (VEGF-A, PlGF, ANGPT1, KDR, FLT-1, and TEK) in C1q-stimulated cells.

Results

OVECs were characterized by immunofluorescence and cytofluorimetric analysis for panendothelial markers and cells resulted 95% positive for endothelial markers and negative for epithelial markers, indicating that we isolated an almost pure population.
Both migration and wound healing assays indicated that C1q was able to induce a pro-migratory phenotype and enhanced OVECs' ability to form capillary-like structures.
To understand whether C1q was able to modulate the angiogenic process at gene level, RT-qPCR was performed onto C1q-treated cells. Results showed that it was able to increase the gene expression of the main pro-angiogenic factors and their receptors.

Conclusions
We were able to isolate an almost pure endothelial population from ovarian biopsies and C1q could induce a pro-angiogenic phenotype onto OVECs.

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