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***In vitro* formation of follicle like structures from aggregations of human ovarian cells sorted using the external epitope of DDX4.**

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**Background**

The existence of egg precursor cells (EPCs) also called Oogonial stem cells (OSCs), has been reported in the adult human ovary challenging the paradigm that the adult female germ cell population has no capacity for regeneration. A population of cells identified as EPCs express the germline marker DEAD-box helicase 4 (DDX4). These cells can be isolated from adult human ovarian cortex by antibody-based Fluorescent Activated Cell Sorting (FACS) exploiting an extracellular epitope of DDX4. FACS sorting also allows DDX4-negative (DDX4-ve) cells to be isolated indirectly. The aim of this study was to determine if DDX4+ve cells could form oocyte/follicle structures when combined with non-germ line cells (DDX4-ve) *in vitro*.

**Method**

Cell suspensions from n=5 post-mortem human ovarian samples (age 29-40 years; mean 35.6 +/- 1.9) were separated by FACS using a proprietary conjugated monoclonal anti-DDX4 antibody. Aggregations of DDX4-ve with DDX4+ve cells were established and cultured individually.

Putative follicle structures (PFSs) approximately 100µM in diameter were observed within the aggregates during the culture period. PFSs were isolated from aggregates by pin dissection and cultured individually for at

least 12 days before fixation for analysis using light and con-focal microscopy.

## **Results**

184 aggregates were established from 8 individual FACS sorts. 46 PFSs (25% frequency) were identified under light microscopy and dissected from aggregates. The mean diameter ( $\pm$ SEM) of PFSs increased significantly during 12 days culture from  $94.5\pm 3.6$  to  $188.3\pm 10.1\mu\text{m}$  ( $p < 0.01$ ).

Haematoxylin and eosin and immunofluorescent staining of isolated PFSs indicated formation of multicellular structures of cuboidal cells organised in a multi-laminar concentric pattern surrounded by an actin-rich basal lamina, around a single putative oocyte cell.

## **Conclusions**

These preliminary data are the first report of the *in-vitro* formation of structures developing from EPCs with the morphological characteristics of ovarian follicles. The physiological relevance of these structures is under investigation.