Abstract content and format:

Title: 25 words

Carbohydrate metabolism profile during oocyte final maturation reveals culture induced aberrations in *in vitro grown* and matured mouse antral follicles

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B: if randomized, identify the trial as such in title
maximum number of words: 25

Study question

C&B:

Are there significant differences in carbohydrate metabolism trends between *in vivo* and *in vitro* grown mouse antral follicles during oocyte final maturation?

maximum number of words: 25

Summary answer

Glucose metabolism characterization during GV to MII transition revealed altered metabolic patterns mainly in cumulus cells of *in vitro* grown and matured mouse antral follicles.

C&B: The main conclusion. A single sentence, this should be limited to the primary results of the study,
without any discussion of their implications
maximum number of words: 25

What is known already

For some cancer patients fertility restoration is dependent on using efficient *in vitro* follicle culture systems. As human donor ovarian tissue available for research is limited, establishing such culture systems relies on data generated from animal models. The culture system previously developed in our laboratory supports *in vitro* growth of mouse preantral follicles with good oocyte maturation rates but lower developmental

competence compared to *in vivo* grown oocytes. Tracking and comparing the metabolic changes after meiotic maturation in *in vitro* and *in vivo* follicles could serve as a screening tool for improving culture conditions and identifying metabolic quality markers.

C&B: One or two short sentences
maximum number of words: 100

Study design, size, duration

Mouse secondary follicle culture was performed. *In vitro* grown oocytes, their corresponding cumulus (CC) and granulosa cells (GC) were collected from antral follicles, at germinal vesicle stage (GV) on day 9, and at metaphase 2 (MII) on day 10, after hCG/EGF stimulation. *In vivo* age-matched controls were obtained after intraperitoneal injections with eCG for GV, or with eCG and hCG for MII. *In vivo* GC after ovulation were not included.

B: Cross sectional – control versus treatment, longitudinal –time-course, age-course.
Numbers of
treated/controls, treatment duration, sampling procedures
maximum number of words: 75

Participants/materials, setting, methods

Glucose metabolism trends were compared during final maturation between in vitro grown antral follicles and their *in vivo* controls. Follicles that failed to resume meiosis in vitro were also included.
Enzymatic spectrophotometric assays were used to measure glycolysis, pentose phosphate pathway (PPP), tricarboxylic acid (TCA) cycle, and the antioxidant capacity in individual cell types. Pools of 5 oocytes and corresponding somatic cells were collected, from 3 independent experiments. Unpaired *t*-test was performed with significance when $p<0.05$.

B: General approach used eg cell/tissue culture/transfection, animal
treatments/models, transgenesis.
Species, ages, gender, cell type. Methods and endpoints used – eg hormone, cytokine, growth factor
measurements, cell numbers/proliferation, tissue morphology/composition, FACS, immunohistochemistry,
Westerns, quantitative PCR, FISH
maximum number of words: 75

Main results and the role of chance

Important differences were detected between *in vivo* and *in vitro* conditions. GV to MII transition in *in vivo* follicles leads to a metabolic boost in CC as indicated by: i. significant increase in glycolysis, PPP and TCA cycle activity; ii. higher total antioxidant capacity (TAC) ($p<0.05$) and small molecule antioxidant capacity (SMAC) ($p<0.01$). After ovulation, the only significant change in oocytes was an increase in nicotinamide adenine dinucleotide phosphate (NADP+) level ($p<0.01$), possibly due to increased reduced-NADP recycling.

Meiotic maturation triggered no significant differences in any of the metabolic pathways for *in vitro* grown oocytes. Contrary to their *in vivo* controls, in vitro CC showed significant upregulations limited to aconitase, lactate dehydrogenase (LDH) and glutathione-s-transferase (GST) activity ($p<0.05$). *In vitro* GC showed increased G6PDH activity ($p<0.05$), suggesting PPP upregulation.

Significant differences were detected between *in vivo* GV follicles and the *in vitro* failed-to-mature ones. Oocytes from impaired follicles have higher $NADP^+$ levels ($p<0.0001$) than their *in vivo* immature counterparts. CC showed higher phosphofructokinase (PFK), LDH, catalase activity and increased $NADP^+$ ($p<0.01$), TAC and SMAC ($p<0.05$) compared to *in vivo* GV CCs. GCs from failed-to-mature follicles have significantly higher LDH and superoxide dismutase (SOD) activity than *in vivo* GV GC ($p<0.05$).

B: P values, biological gradient, repeatability/robustness, mechanisms identified/involved
maximum number of words: 200

Limitations, reasons for caution:

The altered metabolic patterns described here in *in vitro* follicles during oocyte GV to MII transition are probably the cumulative effects of both growth and maturation *in vitro*.

maximum number of words: 50

Wider implications of the findings

We explored extensively and directly, for the first time, several enzymes and metabolites involved in follicle glucose and redox metabolism in different cell types separately. Understanding of the follicle metabolic requirements is essential for the optimization of follicle culture systems and could lead to development of oocyte quality markers.

B: Agreement/disagreement with literature, resolution of previous disparity, new insights/mechanisms in
disease(s), new therapeutic potential, cell-, species- gender-, or age-implications, relevance to other
systems

maximum number of words: 50

Study funding/competing interest(s)
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C&B – select one of the listed options.