44: PI3K/AKT/MTOR AND HIPPO SIGNALING PATHWAYS INTERACTIONS DURING IN VITRO MICE OVARIAN FOLLICLES ACTIVATION.

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Objective
This study aims to better understand the role of PI3K/Akt/mTOR and Hippo signaling pathways during in vitro spontaneous or induced follicular activation.

Design
Postnatal-day 3 mouse ovaries were cultured in vitro for 4h or 48h. Follicular activation was evaluated after whole organ culture (spontaneous activation), chemotherapy exposure (4-Hydroperoxycyclophosphamide, 4HC, 3µM or 20µM during 4 or 24h) and ovarian cutting (induced activation). The efficacy of a mTOR inhibitor, Everolimus (EVE), to inhibit follicular activation was assessed in the different conditions.

Material and Methods
After 48h of culture, follicular activation and apoptosis were evaluated by histological counting and TUNEL staining. The impact on PI3K/Akt/mTOR and Hippo pathways was evaluated by qPCR and Western Blot.

Results
A moderate increase of growing follicles proportion was observed after 48h while the PI3K/Akt/mTOR pathway genes expression was not modified during spontaneous activation. Exposure to chemotherapy induced a dose dependent increase of Akt-P and rps6-P levels and of apoptosis. Hippo pathway was also affected by chemotherapy with an increase of CNN2 expression after 48h. After cutting, Hippo disruption was confirmed by the increase of CCN2 expression at 4h. Sectioning and chemotherapy exposure induced an increase in the growing follicles proportion compared to fresh ovaries but this difference was not significant compared to spontaneous activation. EVE significantly decreased rps6-P levels and prevented spontaneous and chemotherapy-induced follicular activation. Moreover, EVE was able to prevent the chemotherapy induced CCN2 expression.

Conclusions
In vitro follicular activation was moderated in the mice model and involved both PI3K/Akt/mTOR and Hippo pathways. EVE partially inhibits the follicular activation through a decrease of both PI3K/Akt and Hippo effectors suggesting a cross-talk between these two pathways. Inhibition of the PI3K pathway seems to be a promising approach to regulate follicular activation in vitro and reduced chemotherapy-induced “burn out effect”.

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