Reproduction Abstracts

September 2014 Volume 1
ISSN 2052-1472 (online)

World Congress of Reproductive Biology 2014

2-4 September 2014, Edinburgh, UK

World Congress of Reproductive Biology

published by
bioscientifica

Online version available at
www.reproduction-abstracts.org

P044

Insight into progesterone receptor membrane component 1 action during bovine oocyte meiosis by means of siRNA-mediated gene silencing

Valentina Lodde[1], Irene Tessaro[1], Franca Raucci[1], Federica Franciosi[2], Federica Marchese[1], Silvia Modina[1], John J. Peluso[3] & Alberto M Luciano[1]
[1]University of Milan, Milan, Italy; [2]University of California, San Francisco, San Francisco, California, USA; [3]University of Connecticut Health Center, Farmington, Connecticut, USA.

Introduction
Previous studies suggest that progesterone receptor membrane component 1 (PGRMC1) plays an essential role during bovine oocyte meiosis, since it i) localizes to the centromeres at metaphases I and II and ii) concentrates between the separating chromosomes at ana/telophase I. Moreover, injection of an antibody to PGRMC1 significantly impairs completion of meiosis. The aim of the present study is to expand these findings by using siRNA (RNAi)-mediated gene silencing.
Methods
Cumulus–oocytes complexs were microinjected to deliver PGRMC1 or CTRL-RNAi into the oocytes cytoplasm, kept in meiotic arrest for 18 h with 10 μM cilostamide and then *in vitro*-matured (IVM) for 24 h. After IVM, efficacy in depleting PGRMC1 expression was assessed by quantitative RT-PCR and western blotting. Finally, the oocyte capability to extrude the first polar body (PBI) and the morphology of the MII plates were assessed.
Results and discussion
PGRMC1 expression following PGRMC1-RNAi treatment was significantly reduced by 30%. This was accompanied by a 22% reduction of the oocytes that extruded the PBI ($P<0.05$). Surprisingly, PGRMC1-RNAi treatment did not affect MII plate formation or morphology. However, a significantly higher proportion of PGRMC1-RNAi injected oocytes possessed clumps of DNA scattered throughout the ooplasm in addition to the MII plate ($P<0.05$). This is consistent with PGRMC1 localization at the midbody and with a putative role in cytokinesis. We hypothesize that lower PGRMC1 expression impairs the process of PBI formation. As a consequence, DNA that should be extruded with the PBI is retained in the cytoplasm and degraded.
Funding: FP7-PEOPLE-2011-CIG, contract no.: 303640-Pro-Ovum.
DOI: 10.1530/repabs.1.P044

P045

Maturation conditions do not affect *Myst1*, *Hat1*, and *Sirt1* mRNA abundance in horse oocytes

Irene Tessaro[1], Federica Franciosi[2], Pascal Papillier[3], Ghylene Goudet[3], Rozenn Dalbiès-Tran[3], Cècile Douet[3], Fabrice Reigner[3], Stefan Deleuze[4], Valentina Lodde[1] & Alberto M. Luciano[1]
[1]University of Milan, Milan, Italy; [2]University of California, San Francisco, San Francisco, California, USA; [3]INRA, UMR85 Physiologie de la Reproduction et des Comportements, Nouzilly, France; [4]Université de Liège, Liege, Belgium.

Introduction
We recently demonstrated that *in vitro* maturation (IVM) is associated with defects in histone H4 lysine 16 (H4K16) acetylation in horse oocytes, together with a higher incidence of spindle anomalies and aneuploidy. In the present study we investigated whether maturation conditions can alter the abundance of transcripts involved in histone acetylation and deacetylation. The specific H4K16 acetyl-transferase *Myst1*, the general acetyl-transferase *Hat1* and the specific H4K16 deacetylase *Sirt1* were examined in GV-stage oocytes and after IVM.
Methods
Follicle growth was followed by daily ultrasound scanning in adult mares. When a follicle >33 mm emerged, hCG was injected. After 35 h the IVM oocyte was collected by ultrasound-guided transvaginal aspiration. For IVM, oocytes were collected from follicles 5 to 25 mm and cultured for 28 h with EGF and serum. GV-stage oocytes and follicular cell samples were also collected from follicles 5 to 25 mm. Samples were retro-transcribed using random hexamers and analyzed by real-time Q-PCR. *Gapdh* and *Renilla* Luciferase served as internal and external housekeeping respectively.
Results and discussion
Our study reports for the first time the expression of *Hat1*, *Myst1*, and *Sirt1* in horse oocytes and follicular cells. *Hat1*, *Myst1*, and *Sirt1* were expressed at the same extent in GV-stage oocytes and after maturation, independently from the maturation conditions. Our findings show that the transcript abundance of *Hat1*, *Myst1*, and *Sirt1* does not decrease during horse oocyte maturation, strongly suggesting that IVM does not promote/accelerate the degradation of transcripts, but rather affects H4K16 acetylation machinery at translational or post-translational level (L'Oreal 2012).
DOI: 10.1530/repabs.1.P045

P046

Inhibition of mTOR signaling induces cumulus expansion and stimulates meiotic maturation of oocytes in mice

Yumi Hoshino & Eimei Sato
Tohoku University, Sendai, Japan.

The mammalian target of rapamycin (mTOR) signaling pathway functions as a central regulator of cell growth, proliferation, and survival. We previously reported that during meiotic maturation, the expression levels of mTOR in oocytes remain similar from the germinal vesicle (GV) stage to metaphase II (MII). To investigate the role played by mTOR during meiotic resumption, we cultured murine cumulus – oocyte complexes (COCs) in the presence of mTOR inhibitors. mTOR expression was detected in the cumulus cells. The COCs were cultured for 18 h in a medium containing dbcAMP with or without the mTOR inhibitor PI-103 or rapamycin. We observed cumulus expansion but the oocytes were arrested at the GV stage. These oocytes were then transferred to fresh maturation medium containing FSH with or without an mTOR inhibitor before culturing for 8 more hours. We found premature development of the first polar body in oocytes treated with the mTOR inhibitor. This result suggests that mTOR inhibition induces early progression of oocytes. Further, when GV-stage oocytes were cultured for 18 h in maturation medium lacking FSH but containing the mTOR inhibitor PI-103 or rapamycin, the cumulus cells expanded and the first polar body successfully developed. In addition, we found that the mRNA expression of hyaluronan synthase (HAS) in the cumulus cells increased after treatment with the mTOR inhibitor. In conclusion, our data suggest a role for mTOR signaling during cumulus expansion and meiotic maturation in mice. In the presence of an mTOR inhibitor, cumulus expansion occurred and meiotic maturation progressed without gonadotropin stimulation.
DOI: 10.1530/repabs.1.P046

P047

Inhibition of DMRTA2 impairs human female germline development in xeno-grafted ovaries

Marine Poulain[1], Sophie Tourpin[2], Vincent Muczynski[1], Sebastien Messiaen[1], Delphine Moison[1], Clotilde Duquenne[1], Alexandra Benachi[3], Virginie Rouiller-Fabre[1], René Habert[2], Nelly Frydman[1] & Gabriel Livera[1]
[1]UMR967/ Univ P7&P11, Fontenay aux Roses, France; [2]CEA/ INSERM/ Universities P7-P11, Fontenay aux Roses, France; [3]Antoine Béclère Hospital, Clamart, France.

Context
DMRTA2 belongs to a family of genes coding for proteins containing a DM-domain that are conserved among vertebrates and widely involved in gonadal differentiation. We recently identified *Dmrta2* gene expression through transcriptome analysis performed in murine female embryonic germ cells and retrieved its expression in human fetal ovaries. The role of *DMRTA2* is poorly documented and we thus conducted this study to clarify its implication in human fetal ovaries.
Material and methods
Human fetal ovaries (8 – 11 weeks post fertilization) were harvested from material available following legally induced abortions and were grafted in two immunodeficient NMRI:Nu/Nu mice. Recipient mice were treated with siRNA targeting specifically the sequence of human *DMRTA2*.
Results and discussion
The xenograft model did recapitulate all the stages of female germ cell development with no overt change when compared to ungrafted ovaries. After 10 days of treatment with siRNA, we observed of robust inhibition of *DMRTA2*. This inhibition did not alter germ cell density or apoptosis. RT-qPCR and immunostaining analyses indicated that the expression of markers of undifferentiated germ cells was unchanged while the expression of markers of differentiating, pre-meiotic and meiotic germ cells were systematically decreased. This study reveals for the first time the requirement of *DMRTA2* for the normal development of human female embryonic germ cells. *DMRTA2* appears required to allow the differentiation of oogonia shortly prior their entry into meiosis. Additionally, we set up an original model of xenograft that should prove useful for future investigations of the human germline development.
DOI: 10.1530/repabs.1.P047