Addendum: Retinoic acid signaling is critical during the totipotency window in early mammalian development

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In our manuscript, we investigated the potential role of retinoic acid (RA) signaling in the conversion of mouse embryonic stem cells to 2-cell-like cells and in early mouse embryos. It has since been brought to our attention that further scholarly discussion of our findings in early mouse embryos in relation to other previously published work and genetic loss-of-function experiments would benefit a wider readership.

In particular, in the field of early development, there is often misunderstanding in the way that data from genetic knock-outs are interpreted when the products of the genes under study are present, either in mRNA or in protein form, in the maternal germline. The relevant functions cannot be examined from heterozygous mothers as the maternal contribution, in most cases, masks their early zygotic functions[1]. For loss-of-function studies to be meaningful in the early pre-implantation period, a genetic depletion in the mature oocyte is necessary. This is not only due to maternal contribution of mRNA but also proteins and other biomolecules inherited from the oocyte, which provides the embryo with mRNA until at least the 2-cell stage in mice, the time at which zygotic genome activation occurs[2–6]. Genetic analyses of enzymes responsible for RA synthesis show that loss of function for the enzymes does not prevent development past the 2-cell stage but leads to embryonic or postnatal lethality[7–12]. Because these mice do not reach adulthood, any potential role for these enzymes in zygotes and 2-cell stage embryos through maternal deposition of their mRNA or protein in the oocyte cannot be addressed in these mouse models.

Considering this, it is important to appreciate that, so far, all genetic knock-outs targeting RA-producing enzymes and their receptors are zygotic knock-outs. Therefore, a limitation of our study, and that of previous genetic loss-of-function works, is that, until now, none has been performed to specifically deplete members of this pathway in the maternal germline. Indeed, it is notable that mRNAs for all four enzymes synthesizing RA are present in oocytes and zygotes as maternal mRNA[13,14]. Interestingly, though, recent technological advances have made it possible to assess the presence of RA in oocytes and early embryos in mice. While biochemical approaches such as HPLC are challenging in pre-implantation embryos and oocytes, due to the limited amount of material they provide, recent work has indeed provided evidence of the presence of RA in germinal-vesicle and metaphase II stage oocytes as well as mouse zygotes[15].

Collectively, these considerations point towards the need for future work in this area to fully comprehend the molecular regulation and the specific molecules involved in the generation and function of RA signaling in the mature maternal germline and embryos following fertilization.

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