ORC 1 is Required for the First Round of DNA Synthesis in the mouse Zygote

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BACKGROUND

The origin recognition complex (ORC), made up of ORC1, ORC2, ORC3, ORC4, ORC5, and ORC6, is the first complex known to bind to replication origins. This binding initiates the assembly of the pre-replication complex (pre-RC). Pre-RC assembly during G1 is required for replication licensing of chromosomes prior to DNA synthesis during S phase.

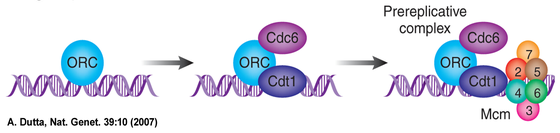


Fig. 1. Regulation of DNA replication by licensing factors. Licensing of an origin of replication by the six-subunit ORC, Cdc6 and Cdt1, leading to loading of the Mcm2-7 complex

ORC1 is part of the complex that binds to origin before ORC1 is absolutely required for zygotic DNA synthesis. However, previous studies have shown that certain cancer cells can replicate DNA in the absence of ORC1, and it was possible that the zygote might replicate DNA without ORC1.

❖ All six ORC proteins have non-chromosomal localization during Anaphase II

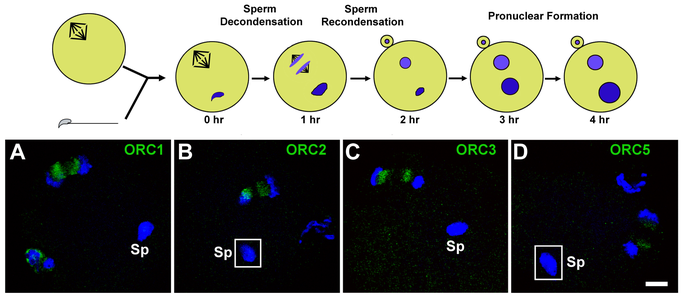


Fig 2. Anaphase II oocytes were isolated and cultured in vitro, then stained for ORC1 and visualized by confocal microscopy. ORC1 (A), ORC2 (B), ORC3 (C), and ORC5 (D) located between two chromosomes at anaphase II.

❖ Conditional Knock out map

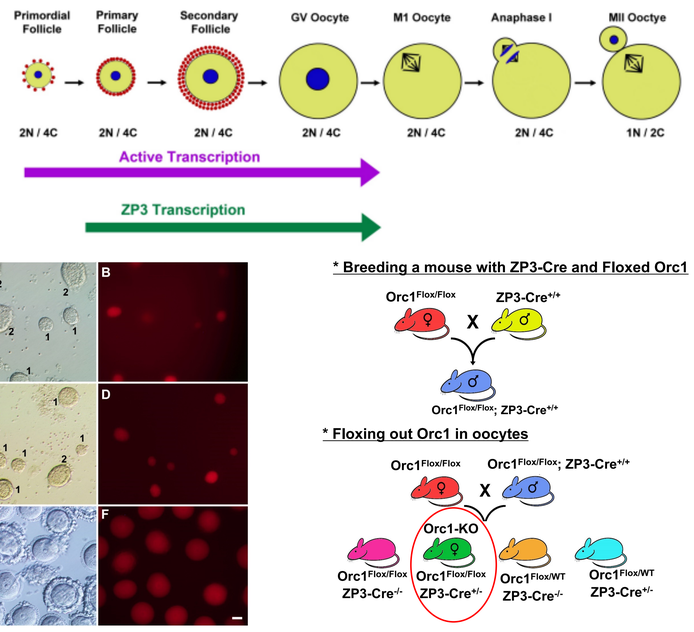


Fig 3. Activation of ZP3-Cre in Oocytes. ZP3-Cre+/+ males were crossed with B6.Cg-Gt(ROSA)26Sortm9(CAG-tdTomato)Hze/J reporter mice, which produce red fluorescent protein (RFP) when Cre is expressed, to verify the activation of Cre during oogenesis.

RESULTS

❖ Orc1 conditional knock oocytes all have Orc1 loss

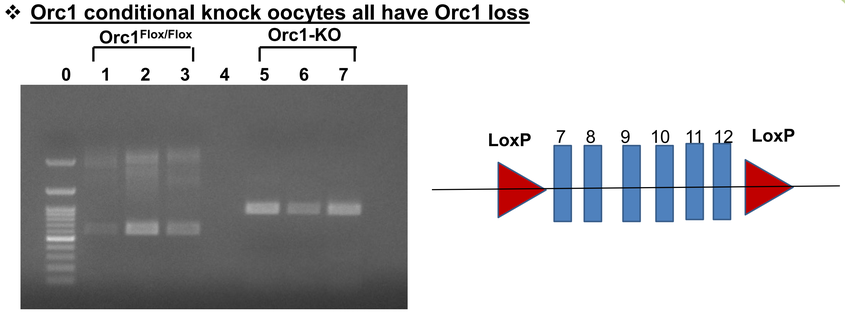


Fig 4. Single MII oocyte PCR for Orc1 $^{flox/Flox}$ and Orc1-KO. Oocyte PCR was performed on MII oocytes from Orc1Flox/Flox and Orc4-CKO mice using the **ORC1 $^{flox/Flox}$ –F, ORC1 $^{flox/Flox}$ –R,** KO-F, and KO-R primers. These primers amplify different sized products for each genotype (Orc1Flox/Flox, 810bp; Orc1-CKO, 964bp)

❖ Orc1-KO GV oocytes to MII stage progression

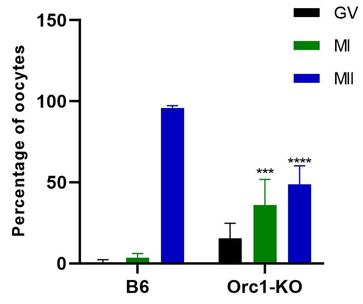


Fig. 5. Number of oocytes and developmental stage for 6 Orc1-CKO mice. Orc1-CKO GV oocytes were isolated and cultured in vitro for 24 hr. The stage to which each oocyte progressed was recorded.

❖ Development of Orc1-KO MII oocytes

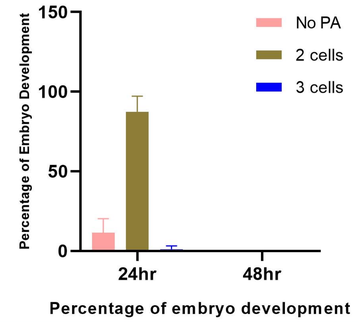


Fig. 6. Progression of activated *Orc1*-KO MII Oocytes. MII oocytes obtained from *Orc1*-KO mice were activated with $SrCl_2$ to progress parthenogenetically and cultured for two days. The stage to which each oocyte progressed was recorded.

❖ Aphidicolin D treatment

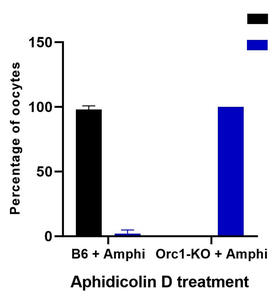


Fig. 7. Activated Orc1-CKO oocytes progress to the two-cell stage without DNA synthesis. B6 and Orc1-CKO MII oocytes were activated to progress through embryonic development by treatment with SrCl2, supplemented with or without aphidicolin (DNA polymerase inhibitor) treatment. The stage to which each oocyte progressed was recorded after culture 24 hr.

❖ Orc1-KO female is not fertile

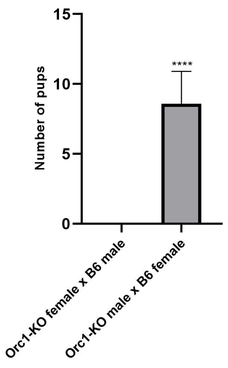


Fig. 8. Female *Orc1* KO mice are infertile, but males are fertile. No pups were obtained when female *Orc1* KO mice were mated with normal B6 males (wt), but male *Orc1* KO mice were able to sire pups when mated with normal females.

❖ Orc1-KO one cell and two cell of DNA replication using MII PA

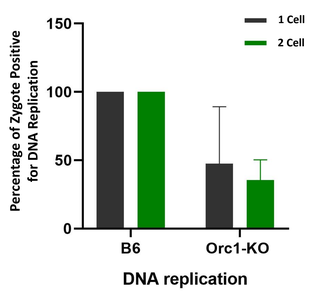


Fig. 9. Activated *Orc1*-KO MII Oocytes underwent DNA synthesis but arrested at two cell stage. MII oocytes obtained from *Orc1*-KO mice were activated with $SrCl_2$ to progress parthenogenetically in the presence of EdU supplement. Activated *Orc1*-CKO oocytes progress to the one cell and two-cell stage with decrease of visible DNA synthesis

RESULTS – CON'T.

❖ Conventional IVF Failed to recue Orc1-KO embryo development

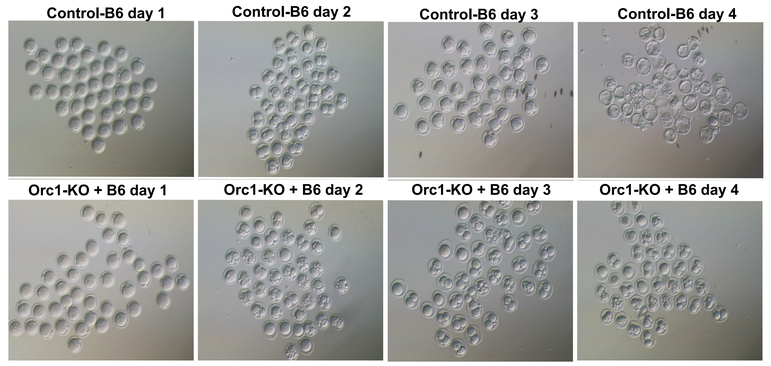


Fig 10. MII oocytes obtained from Orc1-KO mice were used for conventional IVF with B6 sperm and cultured for 4 days. The developmental stage reached by each oocyte was documented. Interestingly, Orc1-KO oocytes did not successfully fertilize with B6 sperm.

❖ ICSI B6 sperm partially recued Orc1-KO embryo development

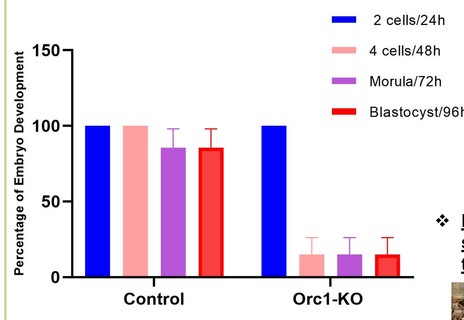
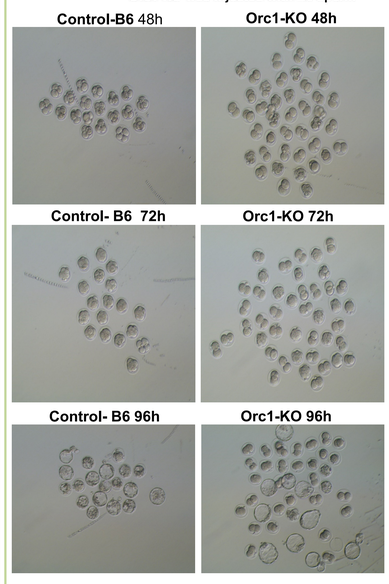


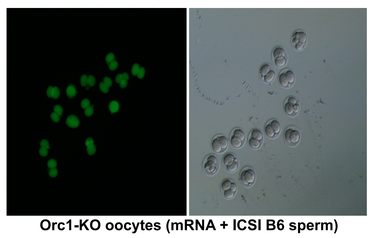
Fig. 11. Progression of *Orc1*-KO MII Oocytes microinjected with B6 sperm. MII oocytes obtained from Orc1-KO mice were injected with B6 sperm and cultured for 5 days. The developmental stage reached by each oocyte was documented. The presence of the Orc1 gene in sperm partially facilitated embryogenesis.



❖ Blastocyst derived from B6-rescued ICSI sperm can produce offspring following transfer.

❖ Pairing of mRNA-Orc1 with ICSI using B6 sperm on Orc1-KO oocytes

MII Orc1-KO oocytes were activated following electrophoresis similar as parthenogenesis. Orc1-KO zygotes experienced arrest at the two to three-cell stage.



Orc1-KO oocytes (mRNA + ICSI B6 sperm)

CONCLUSIONS

- ORC1 is essential for full DNA replication in the zygote and is particularly crucial for progression beyond the 2-cell stage. ORC-1 is necessary for DNA synthesis in both one-cell and two-cell zygotes.
- ICSI can partially rescue ORC1 deletion in the oocyte by providing a new, paternal, *Orc1* gene.

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