Investigating The DNA Damage Response During Oocyte-To-Embryo Transition In Mouse Models

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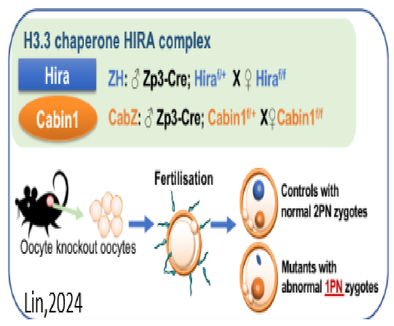
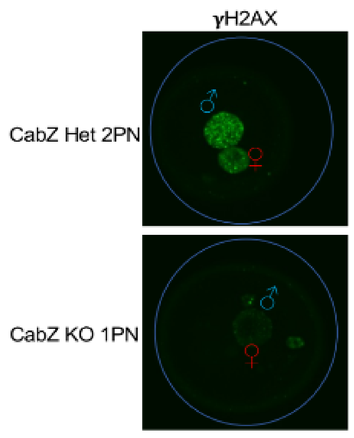
Background

- Cabin1 forms part of the HIRA chaperone complex and is a model of female infertility in knockout mice as only one pronuclei is seen compared to two pronuclei normally seen in wild-types (Smith, *et al.,2022).*



- Since the majority of oocytes remain in meiotic arrest during the entirety of the female's reproductive lifespan, they are vulnerable to DNA damage, leading to genetic mutations or miscarriage. However, DNA damage response pathways can occur, allowing the gestation to continue.

- The wild-type Cabin1 mice express γH2AX, which is a DNA double strand break marker, allowing the repair of DNA, whereas knock-outs have been shown to lack this marker (Lin,2024). Mechanisms of the DNA damage response include homologous recombination (HR) and non-homologous end joining (NHEJ).



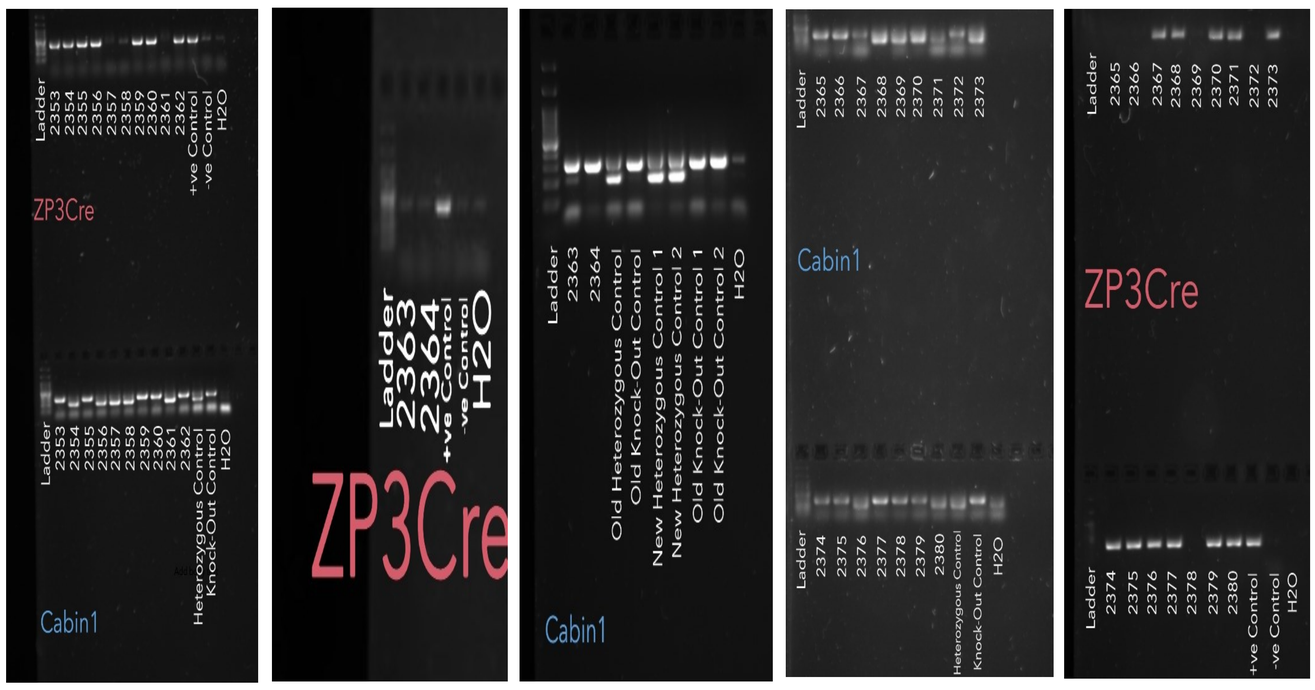
Lin (unpublished)

- Aims of the project: to determine whether inhibiting HR or NHEJ prevents the DNA damage response by measuring γH2AX intensity and comparing this to a solvent control. To assess the importance of these pathways in oocyte-to-embryo transition by monitoring the development of the embryos following the addition of inhibitors of these pathways or a solvent control after parthenogenetic activation or fertilisation.

- This research will allow us to understand the genetic control of infertility better which will help to improve assisted reproductive techniques, aiding many more couples to conceive who were previously unable to do so.

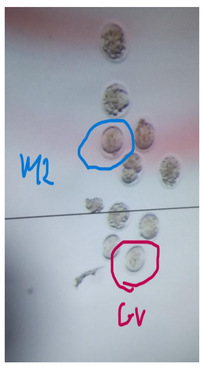
Methods

- Genotyping: PCR was carried out in order to select ZP3Cre+/+;Cabin1f/+ female mice to be used in the experiments as wild-types. DNA was extracted from mouse ear clips and the PCR mastermix was prepared. Different samples were added to the mastermix, along with relative controls and ran on the corresponding PCR programme. Agarose gel electrophoresis was then carried out on the samples.



Agarose gel electrophoresis results carried out for the ZP3Cre and Cabin1 genes on female mice.

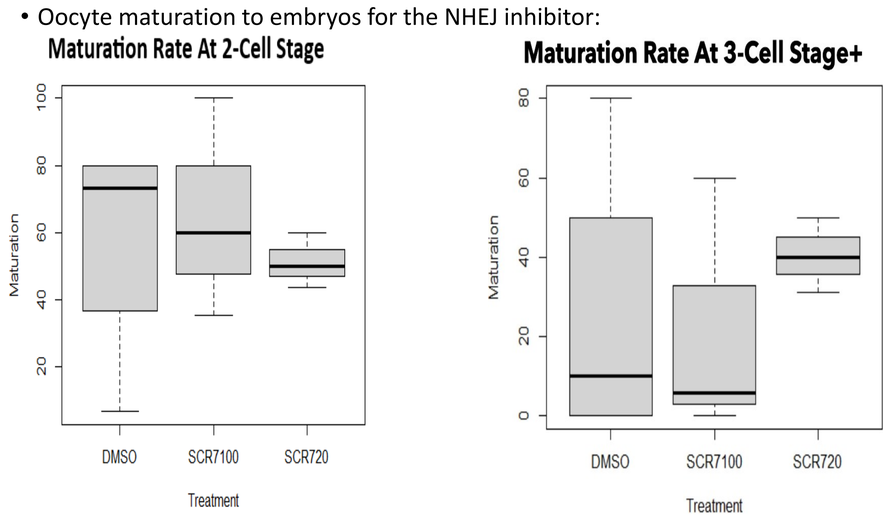
- Oocyte culture: the ovaries of wild type mice were isolated, follicles punctured and the cumulus cells were stripped from germinal vesicle oocytes. The oocytes were incubated in M16 overnight.
- The following day metaphase 2 oocytes were selected and sorted into different treatment groups: DMSO (solvent control), SCR720µmol or SCR7100µmol (the NHEJ inhibitor), each containing parthenogenetic activation media, where they were left to incubate for 5 hours. KSOM drops were prepared and also incubated for this time. Then the oocytes were transferred to KSOM and their development into embryos was monitored over a few days.



An example of the oocyte selection process, where M2=metaphase 2 oocytes were selected and GV=germinal vesicle oocytes were discarded.

Results

- Oocyte maturation to embryos for the NHEJ inhibitor:



- Anova analysis showed there were non- significant differences in the maturation rate at the 2-cell stage and 3 cell plus stage between each treatment group.

Mouse embryos on day 3 of incubation. Results were considered significant where p<0.05.

Conclusions and Limitations

- Although oocytes matured to the 2- cell stage the most under the solvent control, this was not significantly more than the different NHEJ inhibitor concentrations. Interestingly, of these embryos the SCR720µmol treatment was the most successful at progressing to 3 to 4 cells although the differences were insignificant.
- This study showed there was no evidence that blocking non-homologous end joining prevents DNA damage repair in oocyte-to-embryo transition which suggests this pathway is not that important in restoring early embryonic viability.
- Limitations: due to the amount of training required we ran out of time to do experiments for the HR inhibitor. The ovarian dissection was time consuming and few oocytes became M2 so there was a lack of samples for the different treatment groups, meaning experiments needed to be repeated in order to make the results more reliable. Some of the techniques were technically demanding such as the use of the stripper pipette and risk assessment prevented the use of the mouth pipette which also limited the amount of data gathered.

Key Points

- NHEJ does not appear to be important in the DNA damage response in early oocyte-to embryo transition.
- Techniques learned: ovarian dissection, oocyte isolation, tissue culture, PCR, agarose gel electrophoresis.
- Limited by: requirement of training to get practise, number of animals, risk assessment, number of oocytes getting to the M2 stage.

References

Lin C-J (2024). Unpublished.
Smith R,Susor A,Ming H.Tait J.Conti M,Jiang Z & Lin CJ (2022). “The H3.3 chaperone Hira complex orchestrates oocyte developmental competence.” Development