MicroRNAs as Predictive Biomarkers: Assessing Embryo Quality and Aneuploidy

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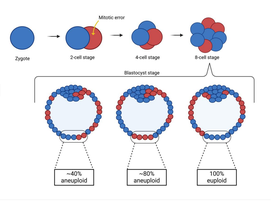
BACKGROUND

- Worldwide, about **1 in 6 couples** will experience infertility.[1]
- In Canada, **28,475 patients** sought fertility treatments in 2023, resulting in approximately **26,525 embryo transfers.**[2]
  - Assisted reproductive technologies have an **average success rate of only 30%**.[2]

CHOOSING THE BEST EMBRYO

- **Morphological grading** involves assessing key criteria like blastomere size and fragmentation under a microscope.
- **Pre-implantation genetic testing (PGT)** requires a biopsy of blastomeres to screen for genetic abnormalities, such as monogenic diseases (PGT-M) or aneuploidy (PGT-A).
- **Aneuploidy** is the most common genetic abnormality found in humans.
  - Leading cause of failed implantation, pregnancy loss, and congenital birth defects.[3]
  - Early mitotic errors can create **mosaic embryos** with aneuploid and euploid blastomeres.



RATIONALE:

Due to the presence of **mosaicism**, PGT-A testing may not reveal the full chromosomal status of an embryo.[4,5] We aim to develop a non-invasive genetic testing method that uses **spent blastocyst media (SBM)** to assess embryos' chromosomal status.

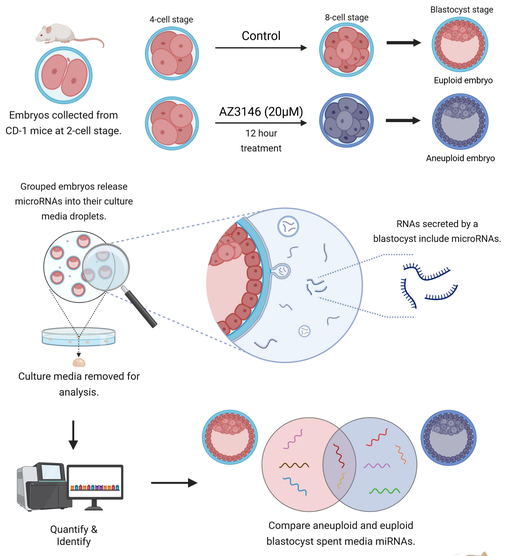
OBJECTIVES:

1. Create an aneuploid mouse embryo model.
2. Isolate and identify miRNA markers in spent blastocyst media.
3. Identify and quantify distinct differences in the miRNA profiles between aneuploid and euploid embryos.

HYPOTHESIS:

We hypothesize that aneuploid mouse embryos will exhibit unique miRNA profiles derived from their spent blastocyst media, compared to euploid controls.

METHODS



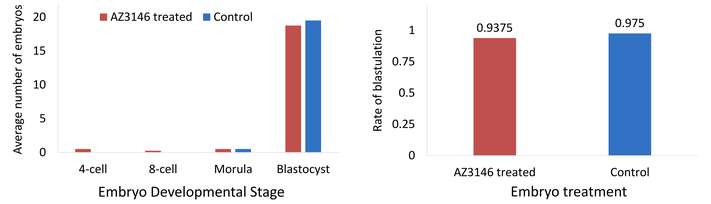
Methods for inducing aneuploidy in mouse embryos using an Mps1 inhibitor drug.
Embryos at the 2-cell stage are collected from 3–4-week-old CD-1 female mice and grown to the 4-cell stage in KSOMaa culture media. From the 4 to 8-cell stage, the embryos are not treated (control) or treated with **AZ3146**, a monopolar spindle 1 kinase (Mps1) inhibitor. AZ3146 disrupts proper chromosome segregation during cell division, leading to aneuploidy. After treatment, embryos are grown to the **blastocyst stage**, after which culture media will be collected for miRNA analysis. To quantify miRNA, **RNA-Seq** will be done for both the embryos and their SBM to search for novel miRNA expression. Aneuploidy will be confirmed through immunofluorescent imaging techniques.

FUNDING

CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada
Children's Health Research Institute
mitacs
Western Research

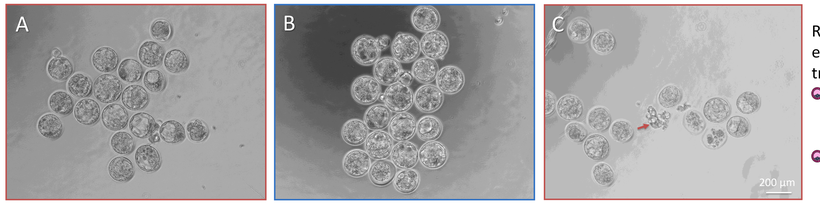
REFERENCES

Blastocyst development after AZ3146 treatment



Developmental potential of AZ3146 treated and control mouse embryos.
Counts were averaged across four droplets (20 embryos in 20μl of KSOM) per treatment group. AZ3146 (20μM) treatment was administered between the 4-8 cell stage, and all embryos were cultured to 104h post-hCG.

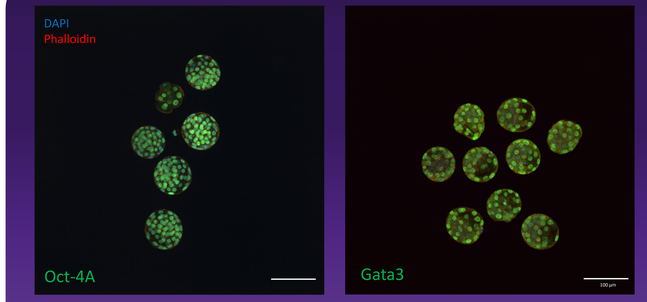
- Blastulation rates ($\frac{number\ of\ blastocysts}{total\ number\ of\ embryos}$) averaged across 4 droplets.
- AZ3146 treated and control embryos had similar rates of blastulation, with no significant differences (t(6)=-1.96, p=0.097).
- AZ3146 treated embryos had more variety in developmental progression, with some 4 and 8-cell embryos in their cohort.



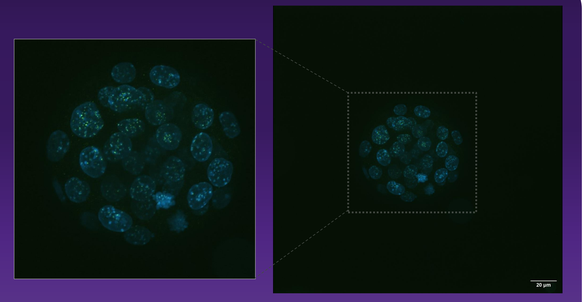
Representative pictures of AZ3146 treated embryos (**A**), control embryos (**B**), and an additional representative image of AZ3146 treated embryos (**C**).

- Degenerative qualities, such as fragmentation and unequal blastomere sizes, can be observed in images A and C. Red arrow indicates degenerate embryo.
- Many AZ3146 treated embryos still managed to progress to the blastocyst stage.

Immunofluorescent Image Analysis



Immunofluorescent lineage markers in mouse embryos.
Mouse embryos at the blastocyst stage (3.5D) stained with trophectoderm marker *Gata3* (1:400, Invitrogen) and inner cell mass marker *Oct-4A* (1:400, Cell Signaling Technology). DAPI blue stains the nucleus and phalloidin red stains for actin filaments. 20x magnification.



Anti-centromere stain on mouse blastocysts.
Mouse embryos at the blastocyst stage (3.5D) stained with a centromere protein-A (1:300, ThermoFisher) marker for chromosome counting to confirm aneuploidy in AZ3146 treated embryos. Green represents the centromeres, blue stains for DAPI. 60x magnification. Image taken by Zuleika Leung.