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Effects of N6-methyladenosine (m^6A) modulators on *in vitro* early embryo development of *Sus domesticus*

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Introduction

N6-methyladenosine is a reversible RNA modification regulated by writers, readers and erasers that is involved in mammalian gametogenesis and embryo development that responds dynamically to ART-related stimuli and heat stress (review by Volsa *et al.*, Hum. Reprod. Update, 3, 181–205, 2026).

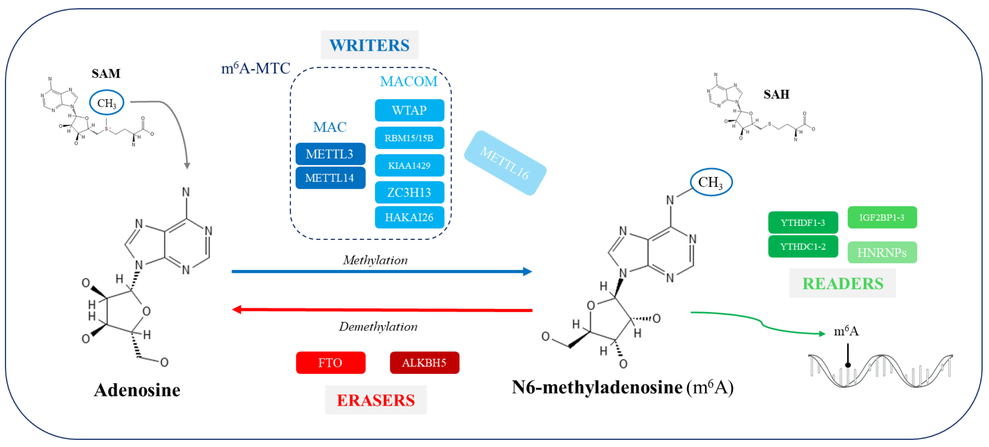

Figure 1. Graphical representation of enzymes. Reprinted from Volsa et *al.*, 2025.

Aim of the study

The main objective of this study was to evaluate the effect of supplementing IVC media with m^6A modulators on early *in vitro* embryonic development in pigs. For this purpose, three m^6A modulators were used: **Cycloleucine**, a competitive inhibitor of methionine adenosyl transferase; **CDIBA**, a non-competitive allosteric inhibitor of METTL3/14 m^6A writers; **FB23**, inhibitor of the m^6A eraser FTO nucleotide recognition lid.

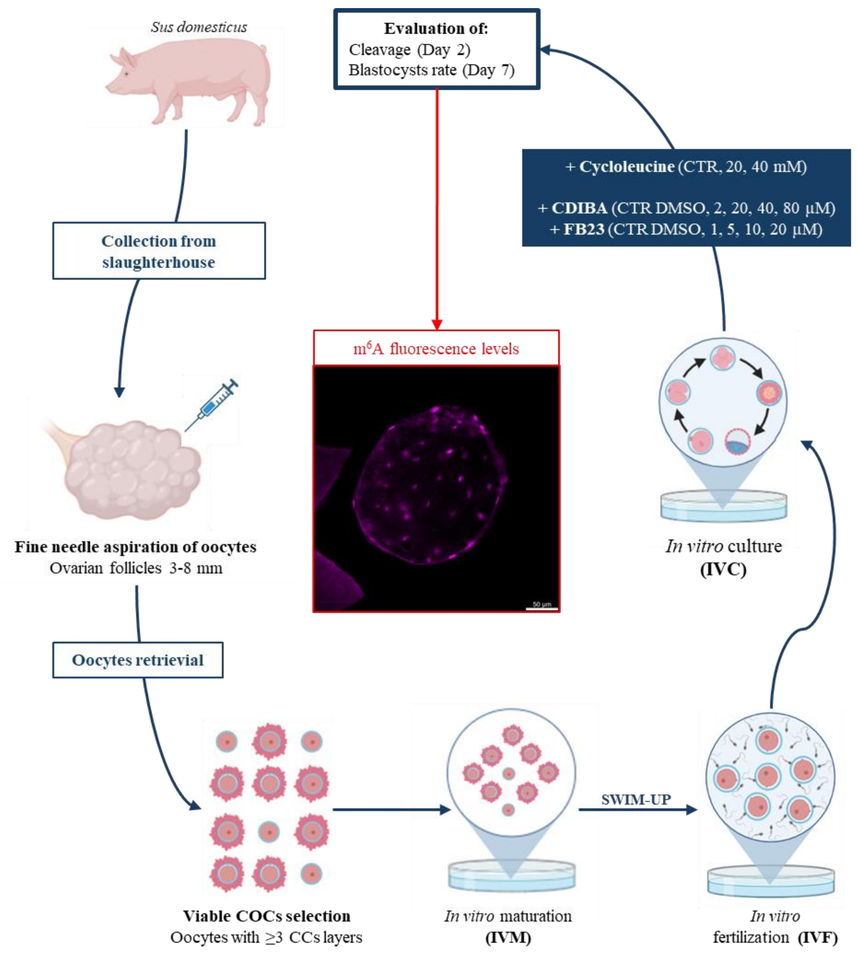

Figure 2. Graphical representation of the experimental design. Created with BioRender.com.

Materials and Methods

Embryos were produced *in vitro* as previously described (Abril-Parreño *et al.*, Environ. Epigenet., 11(1), 2025), using frozen semen from the same male for IVF in 3-4 replicates per experimental group. All the media were purchased from EmbryoCloud (Murcia, Spain). The three inhibitors were supplemented from Day 1 of IVC at different concentrations. Cleavage and blastocyst rates were assessed on Day 2 and 7 respectively, and blastocyst yield was calculated based on the total number of oocytes (B/TOT) and cleaved embryos (B/CLEAV). Statistical analysis was carried out using SPSS and one-way ANOVA followed by Tukey's post-hoc test ($P<0.05$).

Funding

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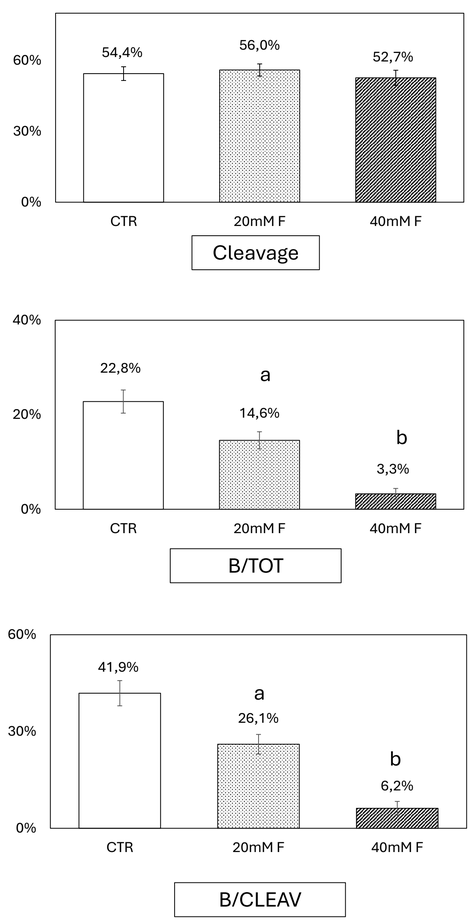
References

- Abril-Parreño L, Lopes JS, Romero-Aguirregomezcorta J, Galvao A, Kelsey G, Coy P. Deciphering differences in DNA methylation and transcriptome profiles of oocytes from pigs with high and low developmental competence. Environ Epigenetics 2025;11:dvaf018.
- Volsa AM, Hoffmann ER, Ross PJ, Canovas S. Epitranscriptomic modifications in embryonic development: insights into natural and ART-induced mechanisms and implications. Hum Reprod Update 2025:dmaf032.

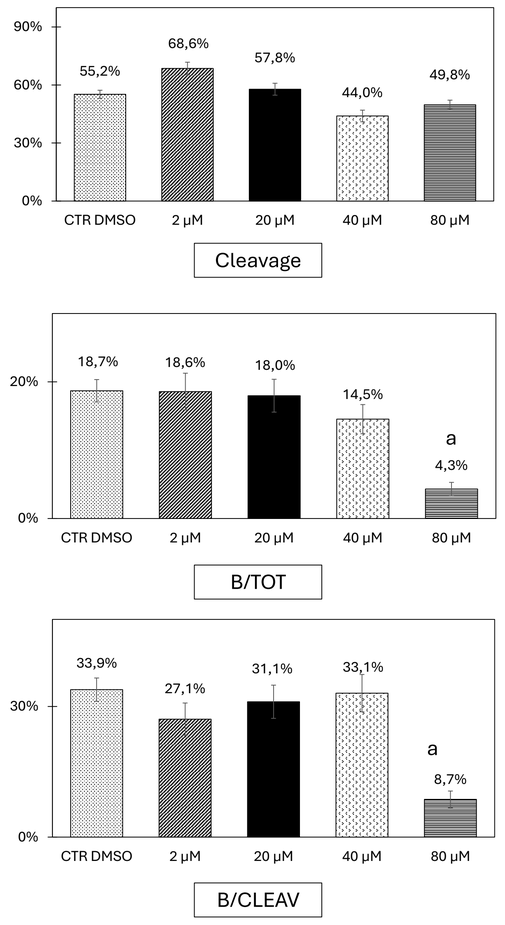
Results

Cycloleucine



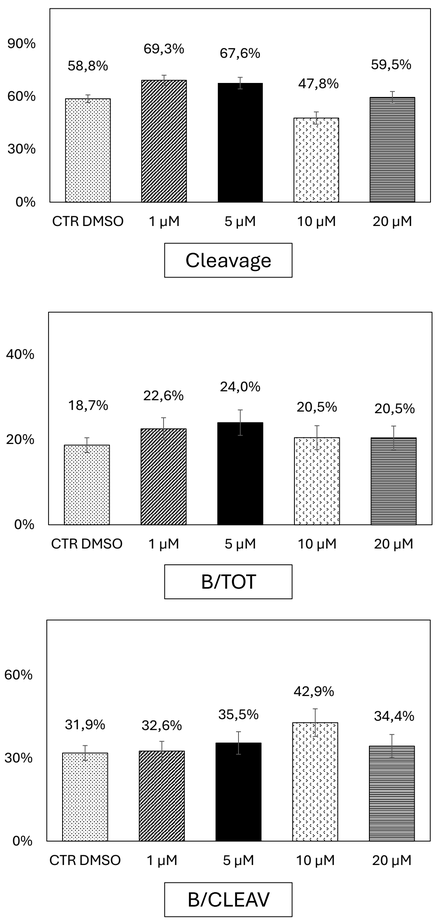
Cycloleucine (CTR, 20, 40 mM) did not affect cleavage rate, but blastocyst formation was reduced by the treatment.

CDIBA



CDIBA (CTR with DMSO (as vehicle), 2, 20, 40, 80 µM), the highest tested concentration - 80 µM- also reduced blastocyst formation.

FB23



FB23 (CTR with DMSO (as vehicle), 1, 5, 10, 20 µM) showed similar blastocyst yield for B/TOT and B/CLEAV.

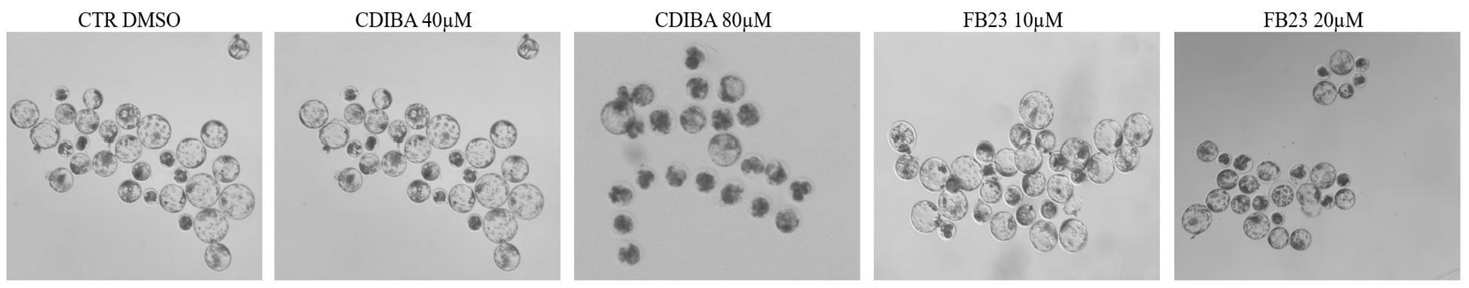


Figure 3. Porcine blastocyst at Day 7 according to each experimental group.

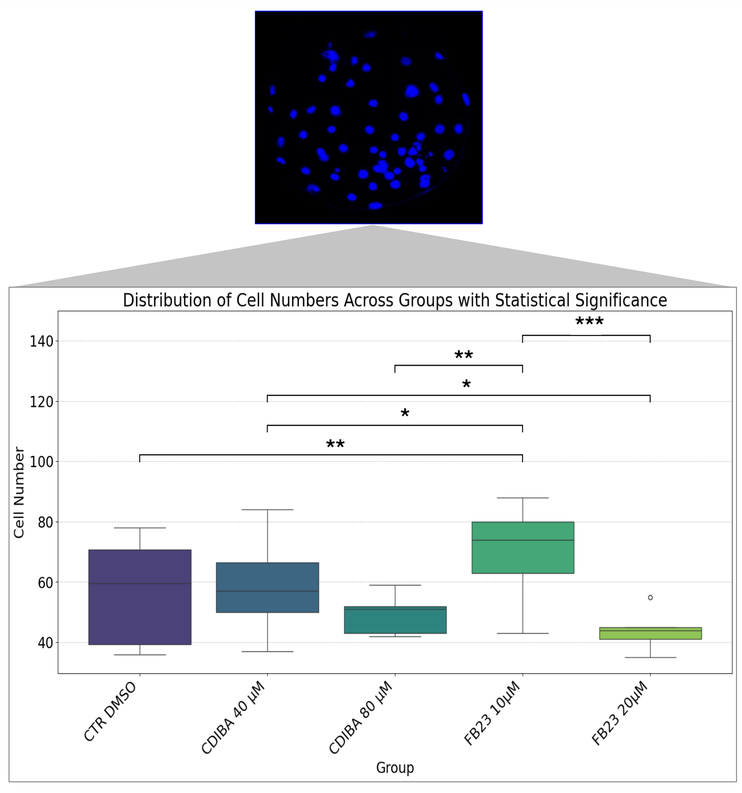


Figure 4. Total cell number in porcine Blastocysts on Day 7 (* for $P<0.05$, ** for $P<0.01$, *** for $P<0.001$). One-way ANOVA followed by Tukey's post-hoc test performed by Python.

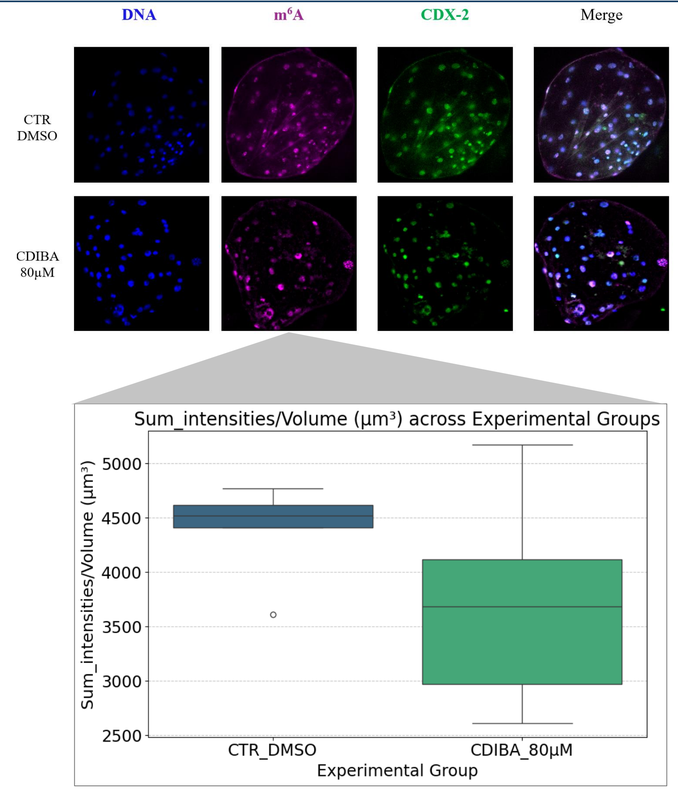


Figure 5. Preliminary data on the differential immunostaining of m^6A of Control DMSO (CTR_DMSO) and CDIBA 80µM (CDIBA_80µM) groups ($P=0.204$). One-way ANOVA followed by Tukey's post-hoc test performed by Python.

Discussion & Conclusions

In summary, m^6A modulators Cycloleucine and CDIBA reduced blastocyst yield in porcine IVF-derived embryos, whereas FTO inhibition by FB23 had no detectable effect within the tested range, indicating that m^6A writing is critical for blastocyst development *in vitro*. Significant differences were observed in the blastocyst cell number between the different experimental groups; while, the m^6A fluorescence intensity did not reach statistical significance ($P=0.05$). Further experiments with a larger sample size would be required to determine whether these preliminary data reflect a biological effect.