Appendix A:

Detection of Development-Specific MicroRNAs in Rabbit Embryos and Culture Media: A Potential Biomarker Approach for Embryo Quality Assessment

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Supplementary Table S1A-1B; Supplementary Figure S1-2; Supplementary Movie

Supplementary table S1.A: List of rabbit embryos cultured in group

Sample Identification	Sample type	Number of cultured embryos	Age	Developmental stage
A_II_e; A_II_m	embryo	5	4-day-old	blastocyst - stage 2
F1_m	medium	5	6-day-old	hatched blastocyst - stage 1
F3_m*	medium	5	6-day-old	hatched blastocyst - stage 2

**Reference sample*

Supplementary table S1.B: List of individually cultured embryos and collected culture medium

Sample Identification	Age	Class
A_I_4_e; A_I_4_m	6-day-old	hatched blastocyst - stage 2
A_I_7_e; A_I_7_m	6-day-old	hatched blastocyst - stage 2
A_IV_1_e; A_IV_1_m	6-day-old	hatched blastocyst - stage 3
A_IV_2_e; A_IV_2_m	6-day-old	hatched blastocyst - stage 3
A_IV_3_e; A_IV_3_m	6-day-old	hatched blastocyst - stage 3
A_III_3_e; A_III_3_m	6-day-old	hatched blastocyst - stage 2
B6_m	6-day-old	hatched blastocyst - stage 2
D6_m	6-day-old	blastocyst - stage 2
D5_m	6-day-old	blastocyst - stage 2
D1_m	6-day-old	blastocyst - stage 2
C2_m	6-day-old	blastocyst - stage 2
A7_m	6-day-old	blastocyst - stage 2
E5_m	6-day-old	blastocyst - stage 1
B2_m	6-day-old	blastocyst - stage 1
A3_m	6-day-old	blastocyst - stage 1

Supplementary Table S2: Proposed functions of the examined miRNAs in relation to embryonic development

miR-103	*miR-103:* Stable in porcine liver and uterus, used for normalization (Mahdipour et al., 2015).
	miR-103a-3p: Regulates embryonic stem cell function, potential therapeutic target (Zhu et al., 2025).
miR-181	*miR-181:* Modulates EMT through TGF-β, Wnt/β-catenin, and NF-κB pathways, linked to cancer cell behaviour (Yang et al., 2025).
	miR-181a-5p: Higher in non-developing embryos; associated with oestrogen signalling and implantation; regulates MMP14 affecting cell adhesion and proliferation (Kamijo et al., 2022; Lázár et al., 2018).
miR-191	*miR-191-5p:* Elevated in aneuploid embryos and failed IVF; linked to cell adhesion and successful implantation (Hawke et al., 2021; Kamijo et al., 2022).
	miR-191: Expression varies with embryo viability and pathology (e.g., preeclampsia, diabetes); insulin-regulated; lineage-specific effects in embryoblast vs. trophoblast cells (Mutia et al., 2023; Pendzialek et al., 2019).
miR-24	*miR-24-3p:* Regulates ESC differentiation; inhibits apoptosis via target gene P27; supports follicular health (Mutia et al., 2023; Shi et al., 2024).
	miR-24: Highly expressed in euploid embryos and polycystic ovaries (Rosenbluth et al., 2013; Zhou & Dimitriadis, 2020).
miR-28	*miR-28-3p:* High in good-quality rabbit blastocysts; supports implantation; influences cancer-related genes (Kamijo et al., 2022; Kotarski et al., 2025).
miR-320	*miR-320:* Highly expressed in early euploid embryos; target: ITGB5; linked to vascular development defects when overexpressed (Caporali & Emanueli, 2011; Rosenbluth et al., 2013).
	miR-320a: Higher in non-pregnancy groups; may negatively affect implantation; associated with CDH and lung fibrosis (Kamijo et al., 2022; Timofeeva et al., 2025).
miR-378	*miR-378a-5p:* Influences trophoblast cell invasion and migration in placentation (Mutia et al., 2023).
	miR-378-3p: Linked to oocyte meiosis, blastocyst quality, embryo hatching, and implantation success (Kamijo et al., 2022; Pavani et al., 2022).

	miR-378: Highly expressed in euploid embryos; associated with sperm motility and quality (Rosenbluth et al., 2013; Turri et al., 2021).
miR-92	*miR-92:* Found in polycystic ovaries (Zhou & Dimitriadis, 2020).
	miR-92a-3p: Regulates genes involved in cell adhesion, important for implantation; may serve as a biomarker for embryo viability (Kamijo et al., 2022).
	miR-92a: Highly expressed in euploid embryos; overexpression causes vascular defects (Caporali & Emanueli, 2011; Rosenbluth et al., 2013).

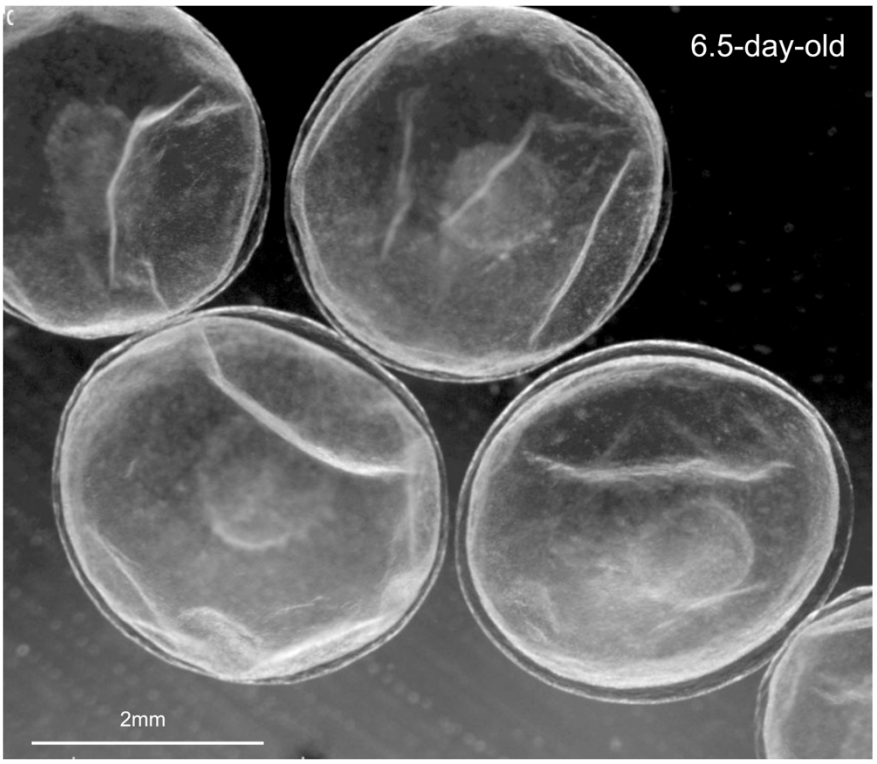
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Supplementary Table S3. Summary of used rabbits and examined embryos

	Experiment 1	*Experiment 2*	Total
Number of used sperm donor males	*4*		**4**
Number of used embryo donor females	4	4	**8**
Number of collected embryos	30	31	**61**
Number of cultured embryos	28	24	**52**
Number of examined blastocysts	28	20	**48**



Supplementary Figure S1:

6.5-day-old (6.5 dpc) rabbit embryos developed *in vivo* in the uterus of a pregnant female and were flushed from the uterus on the 6th day after insemination. We analysed the miRNA expression profile of these embryos using SOLiD sequencing.

Supplementary Movie S1:
The rabbit embryo was carefully retrieved from the oviduct of an inseminated female rabbit two days post-insemination. It was then cultured individually in GT-L medium until it reached the blastocyst stage. The development of the embryo was monitored using CytoSMART Lux2 (Lonza Ltd, Germany) time-lapse video microscopy, which captured images every five minutes in a standard culture environment.