

## Supplementary Material

### **Effects of vitrification of cumulus-enclosed porcine oocytes at the germinal vesicle stage on cumulus expansion, nuclear progression and cytoplasmic maturation**

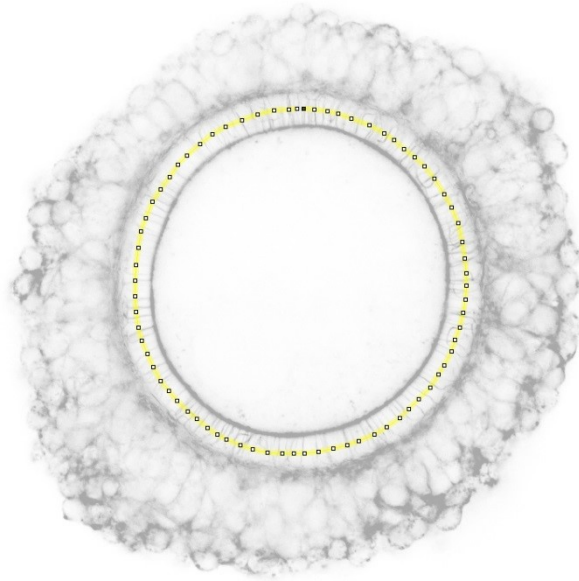
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**Fig. S1.** Quantification of transzonal projections (TZP) in the zona pellucida. After staining of cumulus-oocyte complexes (COCs) with Alexa 488<sup>TM</sup> phalloidin, an image was taken with a laser scanning microscope at the equatorial plane of the oocyte. The image was then converted to 8-bit (grayscale) and inverted by ImageJ software. A circle line (yellow) was drawn in the approximate midline of the zona pellucida perpendicular to the direction of the transzonal microfilaments using the “segmented line” tool. Abundance of TZPs (expressed in arbitrary units) was measured as the mean intensity of gray pixels on the line after setting “Gray Value” as the unit and “Pixel Inverter” as the function in the “Calibrate” menu of ImageJ. To express the relative abundance of TZPs among treatment groups throughout the IVM period, mean TZP abundance in the Control group at 0 h of IVM was considered as 1 for each biological replication of the experiment.

**Table S1. Distribution (%) of different classes of chromatin condensation in GV-stage oocytes at different time points of *in vitro* maturation (IVM)**

Four replications were performed. Data are given as mean  $\pm$  s.e.m. GV stages were classified according to Motlik and Fulka (1976). Different letters denote significant differences between different groups at one time point and within one GV stage. GVII = Germinal Vesicle II; GVIII = Germinal Vesicle III; GVIV = Germinal Vesicle IV; TC = toxicity control

Group	20 h of IVM				30 h of IVM			
	Total	(%)			Total	(%)		
		GVII	GVIII	GVIV		GVII	GVIII	GVIV
Control	125	45.7 $\pm$ 2.3a	54.2 $\pm$ 2.3a	0.0 $\pm$ 0.0	57	13.9 $\pm$ 6.0a	60.1 $\pm$ 4.5	25.9 $\pm$ 2.9a
TC	129	41.7 $\pm$ 5.8a	58.2 $\pm$ 5.8a	0.0 $\pm$ 0.0	85	25.4 $\pm$ 16.5b	65.5 $\pm$ 14.6	9.0 $\pm$ 2.7b
Vitrified	109	27.2 $\pm$ 8.4b	71.4 $\pm$ 7.9b	1.3 $\pm$ 1.3	55	11.3 $\pm$ 5.2a	52.1 $\pm$ 13.8	36.4 $\pm$ 16.5a