Supplementary Material

A prematuration approach to equine IVM: considering cumulus morphology, seasonality, follicle of origin, gap junction coupling and large-scale chromatin configuration in the germinal vesiclep

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Supplemental Fig. 1. Gap junction-mediated cumulus cell-oocyte coupling in compact COCs according to follicle size and season.

Supplemental Fig. 2. Meiotic stage at the end of IVM in not MII oocytes according to cumulus morphology, follicle size and seasonality.

Supplemental Fig. 3. Effect of prematuration on maturation rate.

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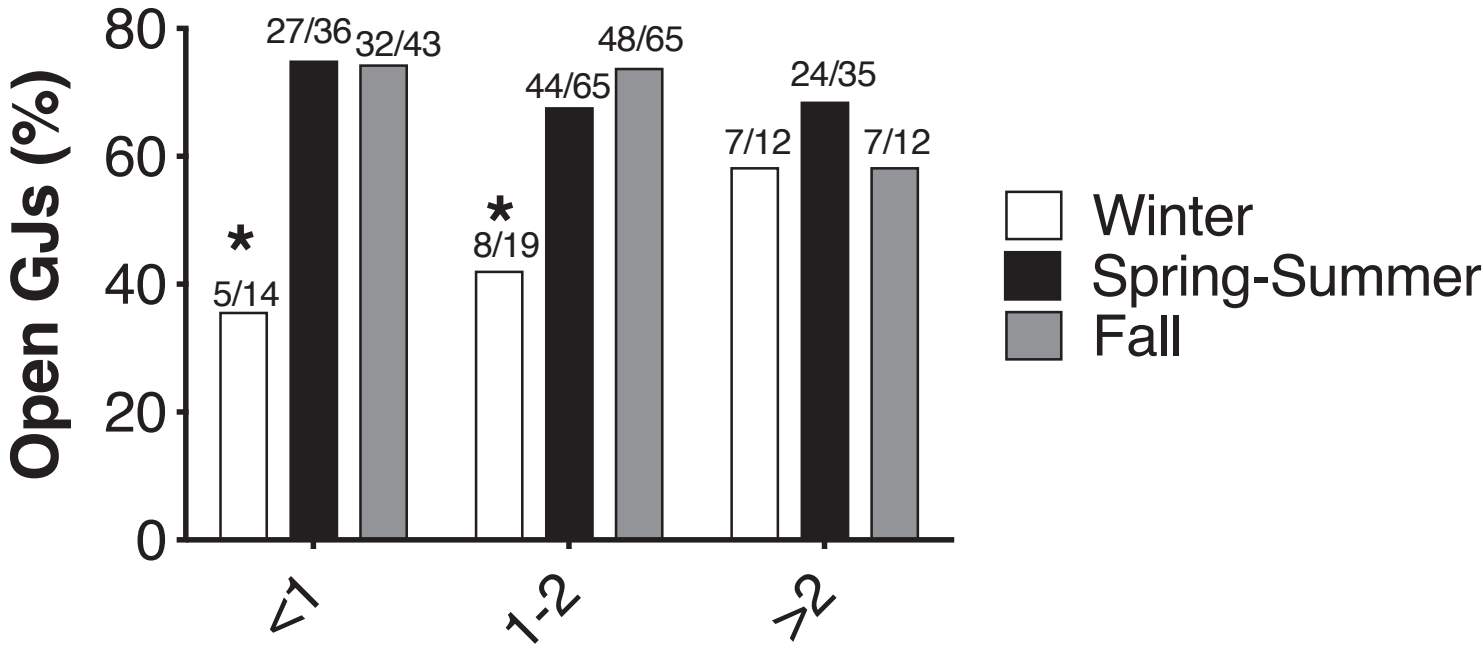
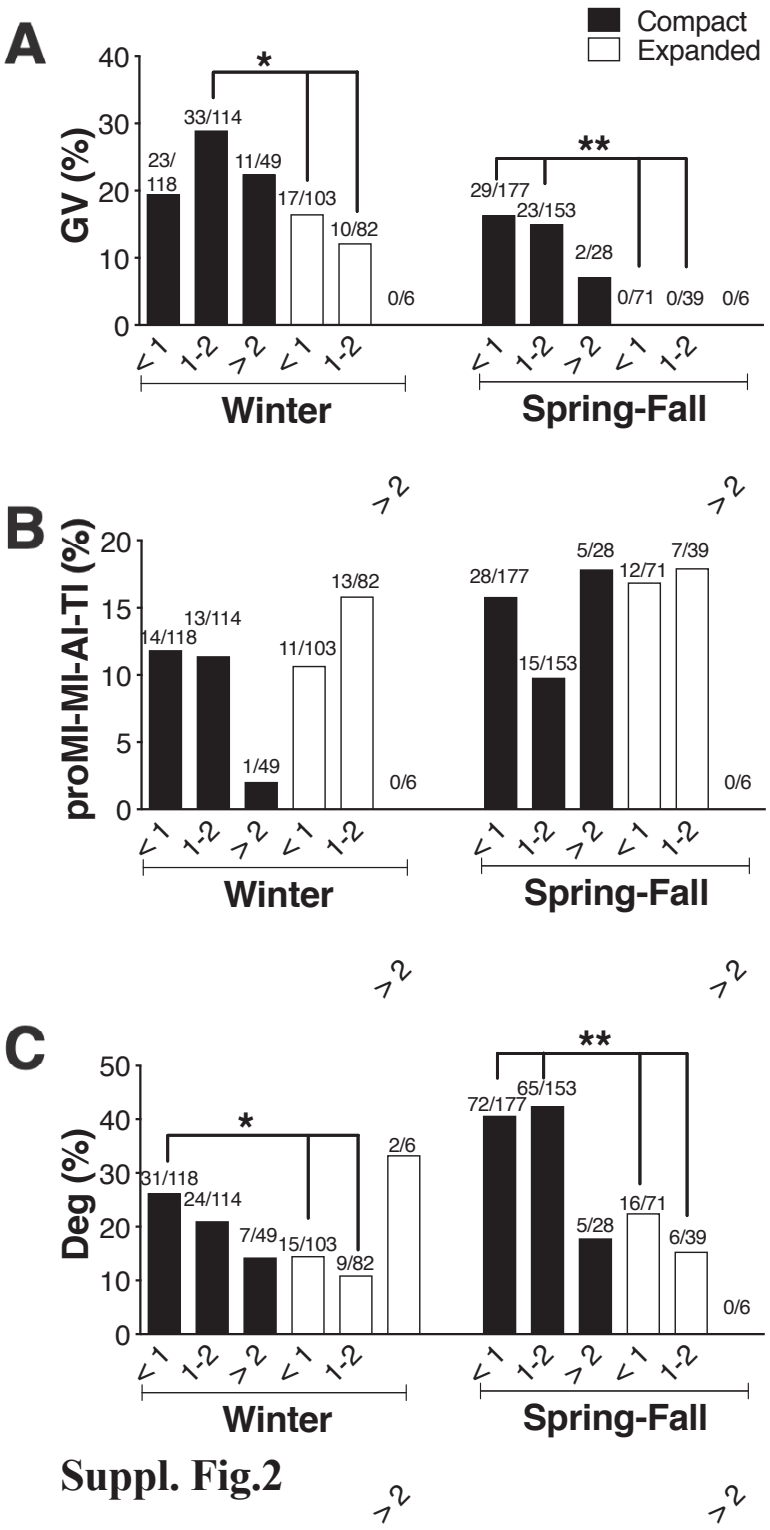
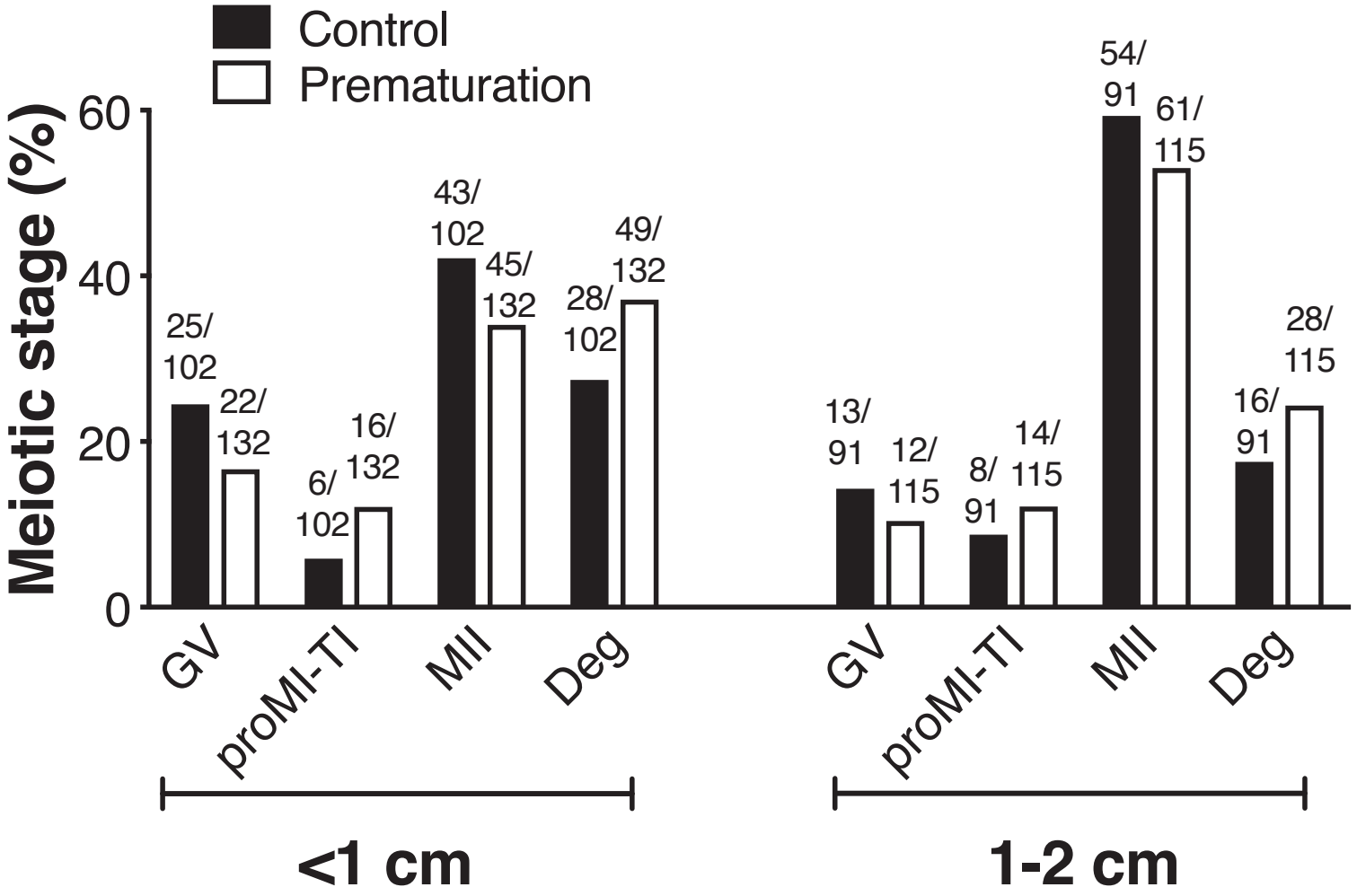


Supplemental Fig. 1. Gap junction-mediated cumulus cell-oocyte coupling in compact COCs according to follicle size and season. Data presented in Fig.1 were re-analyzed considering only the compact class of cumulus morphology. The bar graph represents the rate of COCs with open GJs of the total COCs injected for each follicle diameter/season, across 2-6 independent experiments. Actual number of COCs analyzed (n) is given on top of each bar. Data were analyzed by two-tailed Fisher's exact test. * represents significant differences ($P<0.05$) between COCs collected in winter compared to spring-summer and fall, in the follicles <1 cm and 1-2 cm.



Supplemental Fig. 2. Meiotic stage at the end of IVM in not MII oocytes according to cumulus morphology, follicle size and seasonality.

Compact and expanded COCs collected either during winter or spring-summer-fall (spring-fall) from follicles >1 cm, 1-2 cm and >2 cm in diameter (<1, 1-2 and>2, respectively) were in vitro matured and the stage of meiosis was evaluated by DNA staining. The bar graph represents the percentage of oocytes that at the end of IVM were at the GV stage (A), proMI-MI-AI-TI stages (B) or degenerated (C), across 2-6 independent experiments. Actual number of oocytes analyzed (n) is given on top of each bar. Data were analyzed by two-tailed Fisher's exact test. * and ** represent significant differences ($P<0.05$ and $P<0.01$) in the rate of GV and Deg.



Supplemental Fig. 3. Effect of prematuration on maturation rate. Compact COCs collected from follicles <1 cm and 1-2 cm (<1 and 1-2, respectively) were cultured in prematuration conditions before undergoing IVM. Control COCs underwent IVM directly. The bar graph represents the percentage of GV, ProMI-MI-AI-TI, MII, and deg oocytes at the end of IVM. Actual number of oocytes analyzed (n) is given on top of each bar. Data were analyzed by two-tailed Fisher's exact test. No differences were observed.