Follicle size influences bovine oocyte cytoplasmic maturation and early embryonic development

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Despite recent technological advances and increased use of in vitro embryo production (IVP) in cattle, the outcome of this assisted reproductive technique remains suboptimal. In vitro, up to 80% of oocytes achieve successful nuclear maturation but only 20-30% of oocytes that undergo fertilization result in transferable embryos. Nuclear maturation is a hallmark of oocyte competence, however, during follicular growth and before ovulation, the oocyte undergoes cytoplasmic changes necessary for subsequent embryonic development. Cytoplasmic maturation involves organelle replication and redistribution along with the accumulation of molecules transported from the surrounding cumulus cells into the oocyte via transzonal projections. Oocytes derived from larger follicles exhibit greater developmental potential than those from smaller follicles due to increased exposure to the follicular environment. Meanwhile, in IVP, oocytes are typically collected from follicles ranging from 1-6 mm in diameter. This study investigates the effects of follicle size on oocyte competence as evaluated by cytoplasmic maturation parameters and early embryonic development. Bovine cumulus-oocyte complexes were aspirated from small (<6 mm), medium (6-9 mm), and large (10-20 mm) follicles from slaughterhouse-derived ovaries. Mitochondrial membrane potential and lipid content were measured using fluorescence microscopy along with the distribution of mitochondria and lipids at 0, 12, and 24 h of maturation (n = 305). The number of transzonal projections and cortical granule distribution was also assessed at 0, 12, and 24 h of maturation (n = 160). Embryos from oocytes derived from small, medium, and large follicles were evaluated for cleavage rate on day 3 and blastocyst development on day 8 (n = 412). Mitochondrial membrane potential significantly decreases throughout maturation in oocytes from small, medium, and large follicles ($P = 0.0001$). Lipid content of oocytes from large follicles increases mid-maturation compared to oocytes from small ($P = 0.008$) and medium ($P = 0.078$) follicles but decreases at the end of maturation compared to those from small and medium follicles ($P = 0.037$ and 0.008, respectively). Organelle distribution was described as diffuse, semi-peripheral, or peripheral within the cytoplasm. Mitochondria and lipid distribution in oocytes from small and medium follicles changed from semi-peripheral or peripheral distribution before maturation to predominantly diffuse distribution at the end of maturation. Meanwhile, oocytes from large follicles had continuous diffuse mitochondria and lipid distribution throughout maturation. Oocytes from small follicles had greater transzonal projection count at 0 h of maturation compared to oocytes from medium ($P = 0.002$) and large follicles ($P < 0.0001$). However, the number of transzonal projections did not differ among sizes for the remainder of maturation. Distribution of cortical granules at 0 and 24 h was consistent among follicle sizes, though oocytes from large follicles demonstrated increased transition to peripheral mid-maturation compared to those from small follicles ($P = 0.037$). Furthermore, embryos derived from large follicle oocytes had higher cleavage percentage compared to embryos from small ($P = 0.03$) or medium follicle ($P = 0.04$) oocytes. No significant differences were observed for blastocyst rate from any follicular size ($P = 0.537$). These data suggest that follicle size alone has implications on oocyte cytoplasmic maturation which may affect oocyte competence. Additional

understanding of oocyte competence is required for improved cytoplasmic maturation and improvement of IVP. This work was supported by USDA-AFRI 2022-67015-38938 and SciMed Graduate Research Scholars.