Cumulus-oocyte-complexes of juvenescent mice expand into smaller areas compared to their adult counterparts during in vitro maturation.

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It is well known that with advancing reproductive age, egg quality declines with associated deterioration in fertility outcomes. However, the quality of eggs during reproductive juvenescence is less well understood. Currently, eggs are cryopreserved for fertility preservation in children with cancer and other diseases or treatments that jeopardize their ovarian function. Therefore, it is important to understand the quality of these eggs. Emerging research demonstrates that egg quality in children is inferior to adults with increased aneuploidy rate in the younger age group. A study from our lab demonstrated that mitosis, cell proliferation and cell cycle pathways are down-regulated in cumulus cells (CCs) of adolescents undergoing fertility preservation compared to oocyte donors which represent a group with optimal gamete quality. Cumulus cells are intimately linked with the oocyte, support its metabolism and growth, and undergo expansion prior to ovulation. Cumulus expansion is necessary for successful ovulation and fertilization, and is impaired with advanced reproductive age, which may be indicative of underlying poor gamete quality in the older age group. Our objective in this study was to assess the cumulus expansion in peri- and early post-pubertal mice compared to adults, given that reproductive juvenescence in mice is also associated with increased chromosome abnormalities in the egg. We assessed cumulus expansion *in vitro* in 3 age cohorts: peri-pubertal (Day 16-21), early post-pubertal (Day 22-28), and control adult mice (9 weeks). Cumulus-oocyte-complexes (COCs) were retrieved from the ovaries of Pregnant Mare Serum Gonadotropin (PMSG) injected mice. Individual COCs were imaged pre-expansion, cultured in individual wells in the presence of 10ng/ml EGF and 5% FBS for 12-14 hours, and then imaged post-expansion. Expanded COCs were then treated with hyaluronidase, CCs were removed, and metaphase II oocytes were stained for spindles and chromosomes. Peripubertal mice exhibited significantly smaller COC areas prior to maturation compared to adults (377.6±5.6um^2 (peri-pubertal), 448.6±26.5um^2 (early post-pubertal), 466.8 ±16.5um^2 (adult) p=0.03). These COCs also expanded into smaller areas compared to early post-pubertal and adult mice (683.3±8.4um^2 (peri-pubertal), 776.6±24.6um^2 (early post-pubertal) p=0.0005, 772.2±17.5um^2 (adult) p=0.0008). In metaphase II eggs, chromosome alignment analyses trended more favorably for the adult cohort with a mean of 94.6% appropriately aligned chromosomes on meiotic spindles in this age group, compared to 78.1% in early post-pubertal and 77.4% in peri-pubertal mice. COC area of reproductively juvenescent mice is smaller pre- and post-expansion *in vitro* compared to adults. Additionally, chromosomes tend to be misaligned on metaphase II spindles of juvenescent mice compared to adults. This may be reflective of the suboptimal gamete quality in the younger end of the age spectrum and future research will focus on investigating the implications of this finding for determining the reproductive potential during reproductive juvenescence.

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