
Therapeutic advances over the recent decades have dramatically increased the number of childhood cancer survivors. This success has focused attention on post-treatment quality of life, including fertility, which is frequently compromised by the therapeutic interventions. Currently, cryopreservation of ovarian tissue is the only option available to pre-pubertal girls. However, the effects of cryopreservation on subsequent oocyte and follicular growth remain little understood, due in large part to a lack of knowledge of the early stages in humans. Beginning early during growth, bi-directional signaling between the oocyte and the follicular granulosa cells that surround it regulate oocyte and follicular development. This essential signaling occurs via specialized actin-rich filopodia that project from the granulosa cells to the oocyte membrane. In view of their essential role during folliculogenesis, these intercellular bridges may be a valuable marker of granulosa cell function and follicle quality. We analyzed the morphology of granulosa cell filopodia, as well as the distribution of a protein (MYO10) implicated in the formation of canonical filopodia, in human follicles at early stages of growth. Fresh ovarian tissue was prospectively collected from patients who underwent ovarian surgery. Frozen ovarian tissue was donated by patients who underwent ovarian tissue cryopreservation due to malignant disease. Follicles from primordial to secondary stages were stained using the F-actin binding protein, phalloidin, and anti-MYO10. Confocal microscopy was used to image equatorial optical sections of each follicle. MYO10 expression in the granulosa cells was quantified using Image J. 180 follicles were analyzed and comparisons made between age range (22-30 vs 35-40 years) and fresh vs frozen-thawed follicles. In primordial follicles, actin was present in the oocyte cortex as well as in the squamous adjacent granulosa cells. However, although no filopodia were detectable, some MYO10 foci were present at the interface with the oocyte plasma membrane. As follicles transitioned to the primary stage, filopodia were seen to project from some granulosa cells to the oocyte. This was accompanied by an increase in the number of MYO10 foci. By the late primary stage, a rich network of filopodia extended from the granulosa cells to the oocyte through a visible zona pellucida, named transzonal projections (TZPs). Correspondingly, many MYO10 foci marked the TZP body. Interestingly, whereas some TZPs reached the oocyte plasma membrane others penetrated and connected each other deep into the oocyte. In follicles obtained from 22- to 30-year old women but not in those of 35- 40-year old women, a positive correlation was observed between the distribution of MYO10 in granulosa cells and oocyte diameter. In frozen-thawed follicles from 22- to 30-year old women,
this correlation was no longer observed. Strikingly, large MYO10 aggregates in
the oocyte cytoplasm were observed and significantly increased in frozen-
thawed when compared to fresh follicles. These data suggests a significant
impact of age and cryopreservation on the ability of granulosa cells to
modulate filopodia formation. Additionally, it identifies a method of molecularly
assessing follicle function and quality that may be useful to develop and
improve the existing ovarian cryopreservation methods for pre and post-
pubertal patients.