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The Telomere Theory of Reproductive Senescence

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Reproductive Senescence in Women

Women experience a marked increase in the rate of meiotic dysfunction as they age, resulting in increased infertility, miscarriage and chromosomal non-disjunction, with associated miscarriages and birth defects. Meiotic non-disjunction is the cause of at least 35% of first trimester miscarriages, 4% of stillbirths and 0.3% of live borns [1], and pre-implantation embryos from women at the ends of their reproductive lives exhibit even higher rates (up to 90%) of aneuploidy [2, 3]. The egg must be the major locus of reproductive senescence because donation of eggs from young to older women completely abrogates the effects of age on fertility [4].

Biological Basis of Reproductive Aging in Women

Reduced chiasmata, spindles abnormalities, increased mitochondrial DNA (mtDNA) damage from chronic exposure to reactive oxygen, and late exit from a production line during oogenesis all have been proposed to explain age-related oocyte dysfunction in women. Shortening of telomeres in eggs from older women could explain each of these findings, and provide a unified theory of reproductive aging in women.

The Biology of Telomeres

Telomeres are TTAGGG repeats which cap chromosome ends, prevent end-to end fusions and shorten with each cell division, until they become critically short and cause cell cycle arrest, apoptosis and genomic instability [5-7]. Telomerase, a reverse transcriptase, can reconstitute telomeres, but telomerase activity is quite low from the oocyte to the blastocyst stages of development [14]. Telomeres shorten with age through two mechanisms-replicative senescence and via the effects of reactive oxygen species (ROS) [8]. Once they reach a critically short length, telomeres trigger cell cycle arrest, then senescence.

Telomeres, Chiasmata, Synapsis and Meiosis

In mammals, oocytes initiate meiosis during fetal development and shortly thereafter undergo recombination, then enter a protracted period of meiotic arrest. Chiasmata are formed during fetal life, but resumption of meiosis occurs only during the pre-ovulatory phase of the adult. Chiasmata tether homologous chromosomes during MI, and their deficiency predicts subsequent non-disjunction

and aneuploidy [9,10], presumably because reduced chiasmata provide inadequate counter traction against the pole ward force exerted by the spindle.

Telomere function is critical for chiasmata formation. During early meiosis telomere "bouquets" align homologous chromosomes to facilitate the homology search required for formation of chiasmata [11-13]. When we counted chiasmata, labeled with Mlh1, and measured synapsis, labeled with Scp3, from spreads of fetal, telomerase-null mice with shortened telomeres, we found reduced chiasmata and synapsis. Paucity of chiasmata, coupled with chiasmata-independent effects of short telomeres, would predispose embryos to genomic instability, cell cycle arrest and apoptosis.

Both replicative- and ROS-induced shortening could occur in oocytes. Telomerase is active in early germ cells and late preimplantation stage embryos [14], but oocytes lack telomerase activity, until the blastocyst stage. The prolonged interval between the birth of oocytes and ovulation, up to forty five years in some women, would render them susceptible to shortage by reactive oxygen.

Telomeres and Preimplantation Embryo Development

Telomerase null mice exhibit initially exhibit normal rates of fertilization, development and fertility, but as telomeres shorten across generations, reproductive function deteriorates, with complete sterility just as telomeres reach critically short length.

Telomeres, Metaphase Chromosome Alignment and Meiotic Spindles

Meiotic chromosomes frequently missegregate in human females, leading to aneuploidy, failed implantation and miscarriage [15], and eggs from older women exhibit abnormalities in chromosome congression and spindle morphology. Experiments in adult mice indicate that checkpoints for meiotic chromosome behavior at metaphase-to-anaphase transition are less efficient in females than males [16,17]. We investigated whether telomerase deficiency and/or telomere shortening could influence meiotic progression and found chromosome misalignment and disruption of meiotic spindles at metaphase stages in oocytes from TR-/- mice with short telomeres [18]. Meiotic progression, chromosome behavior and spindle morphology in early generation, TR-/- mice, with long telomeres, resembled wild type mice. Progression from MI to MII stage occurred despite errors in the chromosome alignments at MI, confirming that mammalian female meiosis lacks an efficient metaphase checkpoint control.

The mechanisms underlying chromosome misalignment and disruption of spindles caused by telomere loss are not well understood, but could result from improper homologous chromosome pairing during early meiosis. Telomere loss would disrupt pairing and recombination of homologous chromosomes during leptotene/zygotene stages of prophase I, and prohibit organization and maintenance of functional meiotic spindles, and chromosome alignment at metaphase during meiotic division.

Mitochondrial Dysfunction Shortens Telomeres

Mitochondrial dysfunction and oxidative stress have been implicated in cellular senescence, apoptosis, aging, and aging associated pathologies, including age-related reproductive failure [19,20]. Uncoupling mitochondrial electron transport with FCCP in one-cell zygotes disrupted mitochondrial membrane potential (m) and increased ROS production, measured by CM-H2DCFDA and compromised embryo development in a time- and dose-dependent manner. Telomere length, measured by Q-FISH [20,21,22], demonstrated that mitochondrial dysfunction caused telomere attrition and chromosome. Therefore, ROS production may contribute to telomere attrition and genomic instability. The antioxidant, N-acetylcysteine (NAC), quenches ROS, forms cysteine by deacetylation, and increases glutathione, which itself scavenges free radicals. NAC prevented ROS accumulation, telomere shortening and cell death induced by FCCP. However, NAC did not rescue embryos from telomere attrition, cell cycle arrest and cell death if administered after FCCP treatment.

Telomere length of spare human eggs predicts pregnancy following IVF

Telomere length in chromosome from spare eggs correlated highly with that in their associated first polar bodies (R2=0.98). Telomere maximum (19.3 ± 3.1 k.b. vs. 13.9 ± 3.28 k.b., $p<0.01$) and mean (7.5 ± 1.17 k.b. vs. 6.2 ± 1.69 k.b.) lengths were longer and standard deviation greater (4.4 ± 0.96 vs. 3.5 ± 1.12) in eggs from patients became pregnant, compared to those who failed to become pregnant after IVF. No patients became pregnant if their mean telomere length was less than 6.32 k.b. No other clinical parameters, including patients' age, baseline follicle stimulating hormone (FSH) level, egg number, body mass index, ovarian stimulation protocol, number of previous IVF cycles, diagnosis, or embryo morphology differed significantly between the pregnant and non-pregnant groups in the sample size studied. Decreased egg telomere length portends poor reproductive outcome in infertile women undergoing IVF. Telomere length provided a better predictor of pregnancy outcome following IVF than patient age itself or other clinical parameters, even when telomere length was measured only in spare eggs.

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