Telomere length and reproductive aging

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Introduction

Female fertility declines with age due to the combined effects of both a decrease in the rate of conception and an increase in the rate of pregnancy loss due to aneuploidy (Dorland et al., 1998a). Age-related changes in the human ovary, including depletion of ovarian follicles (Faddy et al., 1992; Faddy, 2000) and a decline in oocyte genomic stability leading to aneuploidy (Hassold and Hunt, 2001), may contribute to this phenomenon. The rate of female reproductive aging displays a large amount of inter-individual variability. This is reflected in the variability in age of reproductive senescence (menopause) that typically occurs anytime between 40 and 60 years of age (Kato et al., 1998; te Velde and Pearson, 2002), as well as in the individual variability in risk of conceiving a trisomic pregnancy (Warburton et al., 2004; Nicolaides et al., 2005). This natural variation in reproductive aging may be the result of environmental and genetic factors that affect individual rates of cellular aging.
Both animal models and human epidemiological studies support the suggestion that longevity is associated with an increase in reproductive lifespan. Mice and flies selectively bred for reproductive longevity have an overall increase in total lifespan when compared with unselected controls (Hutchinson and Rose, 1991; Nagai et al., 1995). Human population studies have reported that higher total fecundity (Manor et al., 2000; Muller et al., 2002), later age at last reproduction (Doblhammer, 2000; Muller et al., 2002; Smith et al., 2002; Helle et al., 2005) and older age at menopause (Snowdon et al., 1989; Cooper and Sandler, 1998; Jacobsen et al., 1999) are positively correlated with longevity. A study of female centenarians found that women living to at least 100 are greater than four times more likely to have had a child while in their 40s than women living at age 73 (Perls et al., 1997). There are several possible explanations for the relationship between longevity and age at menopause: (i) prolonged estrogen exposure associated with later menopause may have a positive influence on life expectancy (Perls et al., 1997), (ii) effective age of the ovary could directly affect longevity (Hsin and Kenyon, 1999; Cargill et al., 2003) or (iii) selective pressures to maximize a woman’s reproductive years by slow reproductive aging may have positively selected for women with slower rates of cellular aging (Perls and Fretts, 2001; Perls et al., 2002).

Telomeres are a marker for cellular aging as their length declines with each cell division. Telomere length exhibits considerable inter-individual variation (Hastie et al., 1990) and may contribute to the observed variability in reproductive aging. Telomere variability may be due to differences in telomere length at conception, telomerase activity during early development, rate of cell division and rate of telomere loss per cell division. Shorter telomeres may limit the mitotic capacity of primordial germ cells during fetal development and therefore restrict the size of the follicular pool (Keefe et al., 2006). Studies examining telomere length and reproductive aging in humans have produced contradictory results in which telomere length has been both positively and negatively associated with different measures of reproductive aging (Dorland et al., 1998a; Aydos et al., 2005; Keefe et al., 2007).

Given the links between reproductive aging and biological aging, and the potential influence of telomere length on oocyte quality, we hypothesized that women who display evidence of premature reproductive aging will have a shorter average telomere length than control women. The objective of this study was to assess telomere length in peripheral blood leukocytes in two groups of women with evidence of premature reproductive aging: (i) patients with idiopathic premature ovarian failure (POF) who experienced menopause before 40 years of age and (ii) women with a history of recurrent miscarriage (RM), and two control groups: (1) women from the general population not selected on the basis of reproductive history and (2) women who had a healthy pregnancy after the 37 years of age and had not experienced any pregnancy loss. This latter group may represent women with potentially slower rates of reproductive aging, as they have not experienced difficulties conceiving or maintaining pregnancy despite a relatively advanced reproductive age.

**Materials and Methods**

**Samples**

Women with RM (N = 95), defined as three consecutive miscarriages of <20 weeks of gestation, were ascertained through the Recurrent Pregnancy Loss Clinic at the Women’s Health Centre of British Columbia. These 95 women had a total of 458 miscarriages, and of those, 167 were karyotyped. Karyotyped miscarriages consisted of 72 diploid losses, 71 aneuploid losses and 24 other anomalies, including polyploidy, sex chromosome aneuploidies and translocations. Of those women with aneuploid losses, there were 32 women who had a single trisomic miscarriage (ST) and 17 women who had multiple trisomic miscarriages (MT). POF patients (N = 34) with idiopathic secondary amenorrhea were ascertained from the POF Clinic at the Women’s Health Centre of British Columbia. POF diagnosis was made based on the absence of menses for at least 3 months and two serum FSH results of >40 mIU/ml obtained more than 1 month apart, prior to 40 years of age. Two control groups were used in this study: Control group 1 (N = 108) consisted of healthy women of reproductive age, ranging from 17 to 55 years, and Control group 2 (N = 46) consisted of women who have had a healthy pregnancy over 37 years of age with no history of infertility or miscarriage. DNA was obtained by standard salt extraction from ~5 ml of blood collected in EDTA tubes. The collection of the DNA samples for this study was obtained after informed consent and approved by the University of British Columbia Clinical Ethics Review Board, approval number CO1-0460.

**Telomere length**

Average relative telomere length was determined by quantitative PCR (qPCR) (Cawthon, 2002). Amplification of the telomeric repeat region was expressed relative to amplification of 36B4, a single copy housekeeping gene on chromosome 12. This telomere to single copy (T/S) ratio is proportional to the average telomere length of the sample, due to the amplification being proportional to the number of primer binding sites in the first cycle of the PCR reaction (Cawthon, 2002). The protocol was performed as described by Cawthon (2002) with several modifications; amplifications were carried out in 20 µl reaction with ~5 ng genomic DNA, 0.5 µM ROX Reference Dye (Invitrogen, Carlsbad, USA) and 0.2 × SYBR Green I nucleic acid gel stain in dimethylsulfoxide (Invitrogen). Samples were run in triplicate on 96-well plates containing a standard curve constructed with reference DNA serially diluted to concentrations from 10 to 0.625 ng. A no-template control and both short- and long-telomere reference samples were run on each plate as quality controls. Dissociation melting curves were run after each sample to ensure amplification of a single species. Replicates of each plate were done to ensure reliable values were ascertained. The values between both runs were significantly correlated, with a correlation coefficient of \( r = 0.49 \) (\( P < 0.0001 \)). To improve the accuracy of our estimates for each individual blood sample, we averaged the values of the two independent measurements. When values were discrepant between the two runs by more than 0.2 SDs, subsequent runs were done and an average of all values was used in further data analysis.

The telomere-specific qPCR assay was validated using DNA extracted from leukocyte cell pellets following flow fluorescence in situ hybridization (FISH) (N = 12) (Baerlocher et al., 2006). There was a strong correlation between the qPCR T/S ratio and the flow-FISH telomere lengths (\( r = 0.96 \)). The strong correlation obtained validates the use of an average measurement of T/S values as an accurate reflection of telomere length. T/S values were converted to kilobases (kb) using the linear equation from this correlation (\( y = 7.25 \times x + 2.50 \)). As expected the y-intercept is at 2.5 kb since the flow-FISH assay was calibrated using Southern blot telomere restriction fragment lengths, which includes ~2.5 kb of subtelomeric repeat (Baerlocher et al., 2006).
Statistical analysis
Rate of telomere decline was determined by linear regression analysis, and one-tailed t-tests were used to determine the significance of the regression because of the a priori hypothesis that telomere length was associated with age. Yearly rates of telomere decline were compared using two-tailed t-tests for comparison of regression slopes. Mean telomere length comparisons between sample groups were determined using pairwise analysis of covariance (ANCOVA) tests to adjust for differences in ages between sample groups.

Results
Telomere length in Control group 1 significantly declined with age ($P = 0.001$, one-tailed t-test) at a rate of 40 bp per year [95% confidence interval (CI) = 14–66 bp], although there was significant variability in telomere length at any given age ($R^2 = 0.081$, Table I, Supplementary Fig. 1). There was also a weak ($R^2 = 0.161$) but significant negative association between telomere length and age in POF patients ($P = 0.01$, one-tailed t-test), but not in Control group 2 or the RM group as a whole. Subsets of the RM group who have experienced ST or MT are of particular interest, as incidence of trisomic pregnancy increases with age, contributing to the age-related increase in RM. There was a weak ($R^2 = 0.130$) but significant relationship between telomere length and age in the ST subset of the RM group ($P = 0.02$, one-tailed t-test) but not the MT subset (Table I, Supplementary Fig. 2). However, in no sample group was the rate of telomere decline significantly different from that of Control group I (two-tailed t-tests for comparison of regression slopes), thus ANCOVA was used to adjust for age effects on mean telomere length for further comparisons of mean telomere length between groups.

Mean telomere length and age-adjusted mean telomere length for each sample group are shown in Table II. Although women in Control group 2 had longer age-adjusted mean telomere lengths than those in Control group 1, this difference was not significant. The RM group had shorter age-adjusted mean telomere length than Control group I (8.46 versus 8.92 kb, $P = 0.0004$) and this was also apparent in comparison with Control group 2 (9.11 kb, $P = 0.02$). However, short telomeres were not specifically confined to the subset of this group that had had either a single trisomy or multiple trisomic pregnancies. Contrary to expectation, age-adjusted mean telomere length in the POF patient group was longer than that in Control group I (9.58 versus 8.92 kb, $P = 0.01$), although this was not significant in comparison with Control group 2.

Discussion
Telomere-specific qPCR was used to assess telomere length in groups of women with a reproductive history suggestive of premature

<table>
<thead>
<tr>
<th>Sample group</th>
<th>N</th>
<th>Age range (years)</th>
<th>Rate of telomere decline (base pairs/year)</th>
<th>$R^2$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>Lower 95% CI</td>
<td>Upper 95% CI</td>
</tr>
<tr>
<td>Control group 1</td>
<td>108</td>
<td>17–55</td>
<td>–40</td>
<td>–66</td>
<td>–14</td>
</tr>
<tr>
<td>Control group 2</td>
<td>46</td>
<td>37–54</td>
<td>–26</td>
<td>–56</td>
<td>107</td>
</tr>
<tr>
<td>POF patients</td>
<td>34</td>
<td>21–50</td>
<td>–98</td>
<td>–178</td>
<td>–17</td>
</tr>
<tr>
<td>RM</td>
<td>95</td>
<td>24–45</td>
<td>–3</td>
<td>–40</td>
<td>40</td>
</tr>
<tr>
<td>Single trisomy</td>
<td>32</td>
<td>24–44</td>
<td>–56</td>
<td>–110</td>
<td>–2</td>
</tr>
<tr>
<td>Multiple trisomy</td>
<td>17</td>
<td>33–44</td>
<td>–23</td>
<td>–150</td>
<td>105</td>
</tr>
</tbody>
</table>

$\text{CI, confidence interval; POF, premature ovarian failure; Control group 1, women from the general population; Control group 2, women who had a healthy pregnancy after 37 years of age.}$

$R^2$ is a measure of the goodness of fit of the regression.

$P$-values are based on a one-tailed test for significance of the regression based on the t-distribution.

<table>
<thead>
<tr>
<th>Sample group</th>
<th>N</th>
<th>Mean age (years)</th>
<th>Mean telomere length</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Raw data (+ SD) (kb)</td>
<td>Age-adjusted (kb)</td>
</tr>
<tr>
<td>Control group 1</td>
<td>108</td>
<td>36.3</td>
<td>8.98 ± 1.15</td>
<td>8.92</td>
</tr>
<tr>
<td>Control group 2</td>
<td>46</td>
<td>41.5</td>
<td>8.99 ± 1.03</td>
<td>9.11</td>
</tr>
<tr>
<td>POF patients</td>
<td>34</td>
<td>35.4</td>
<td>9.61 ± 1.38</td>
<td>9.58</td>
</tr>
<tr>
<td>RM</td>
<td>95</td>
<td>35.8</td>
<td>8.47 ± 0.92</td>
<td>8.46</td>
</tr>
<tr>
<td>Single trisomy</td>
<td>32</td>
<td>36.3</td>
<td>8.80 ± 0.78</td>
<td>8.80</td>
</tr>
<tr>
<td>Multiple trisomy</td>
<td>17</td>
<td>39.3</td>
<td>8.42 ± 0.69</td>
<td>8.52</td>
</tr>
</tbody>
</table>

$P$-values in comparison with Control groups 1 and 2, respectively.

Analysis of covariance ($k = 2$ in comparison with Control group 1 or 2) was used to adjust raw telomere length data by age in comparisons between groups.
Telomere length and reproductive aging

Telomeres are repetitive DNA sequences at the ends of chromosomes that act as protective caps. As cells divide, telomeres become shorter, which can affect reproductive function. Several studies have investigated telomere length and reproductive aging, focusing on conditions such as premature ovarian failure (POF) and reproductive senescence.

### Premature Ovarian Failure (POF)

POF is characterized by the premature loss of ovarian function, often occurring before the age of 40. Several studies have explored the relationship between telomere length and POF. One study found that women with POF had shorter telomeres compared to control women, although this effect was not significantly different in all sample groups, possibly reflecting the limited age range in some groups. Regardless, these findings suggest a different rate of telomere decline than that observed in control populations.

### Reproductive Senescence

Reproductive senescence refers to the decline in reproductive function associated with normal aging. Similar to POF, reproductive senescence is associated with telomere shortening. Studies have shown that telomere shortening occurs in oocytes from women undergoing in vitro fertilization (IVF), and this may impact the quality of embryos.

### Psychological Stress

Psychological stress, including autoimmune diseases, can influence telomere length. Autoimmune conditions are associated with shortened telomeres, which may reflect higher levels of psychological stress. However, telomere length measured in peripheral blood may not necessarily reflect telomere length at the ovary or developing embryo. Further research is needed to clarify the relationship between telomere length and reproductive aging.

### Conclusion

Telomere length and reproductive aging are complex processes influenced by various factors. Future studies should consider the impact of telomere length on reproductive outcomes and explore the role of telomerase activity and telomere maintenance mechanisms in reproductive disorders.

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**Key Points:**
- Telomere length is a determinant of reproductive aging.
- Reproductive senescence and POF are associated with telomere shortening.
- Psychological stress, including autoimmune diseases, can influence telomere length.
- Further research is needed to clarify the relationship between telomere length and reproductive aging.
that RM and POF showed opposite associations with telomere length, and trisomic pregnancy showed no evidence of a consistent association, suggests that these different types of reproductive aging are likely influenced by unique factors. Further studies are necessary to confirm these findings in larger more precisely defined populations, examine the physiological mechanisms that influence both telomere length and reproductive aging and investigate the molecular mechanisms responsible for longer telomere lengths in the POF population.

Supplementary Data

Supplementary data are available at http://humrep.oxfordjournals.org.

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References


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