Reproductive BioMedicine Online (2014) **28**, 624–637

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ARTICLE

Telomere length in human blastocysts

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Abstract This is a retrospective study aiming to assess telomere length in human embryos 4 days post fertilization and to determine whether it is correlated to chromosomal ploidy, embryo developmental rate and patient age. Embryos were donated from patients undergoing treatment in the assisted conception unit. Seven couples took part, generating 35 embryos consisting of 1130 cells. Quantitative fluorescent in-situ hybridization (FISH) measured the telomere length of every cell using a pan-telomeric probe. Conventional FISH on six chromosomes was used to assess aneuploidy in the same cells. Maternal and paternal age, referral reason, embryo developmental rate and type of chromosomal error were taken into account. Chromosomally abnormal cells were associated with shorter telomeres than normal cells for embryos that were developmentally slow. Cells produced by women of advanced maternal age and those with a history of repeated miscarriage tended to have substantially shorter telomeres. There was no significant difference in telomere length with respect to the rate of embryo development 5 days post fertilization. Telomeres play an important role in cell division and shorter telomeres may affect embryonic ploidy. Reduced telomere length was associated with aneuploid cells and embryos from women of advanced maternal age.

KEYWORDS: aneuploidy, blastocyst, human, preimplantation embryo, quantitative FISH, telomere length

Introduction

Telomeres are six base-pair repeats (TTAGGG) found at the ends of chromosomes to protect them from degradation (Chan and Blackburn, 2002). Telomeres participate in processes of chromosomal repair, prevent nonspecific chromosomal recombination and help the chromosome bind to the nuclear matrix (Counter et al., 1992; De Lange, 1992). Prior

http://dx.doi.org/10.1016/j.rbmo.2013.12.010
1472-6483/

to mitotic cell division, cellular DNA is duplicated by the action of a DNA polymerase. This enzyme does not replicate the ends of chromosomes and so telomeres get shorter after each cell division. In normal somatic cells, this shortening contributes to cellular senescence (Blasco et al., 1997). Telomere shortening limits the regenerative capacity of cells and is correlated with the onset of cancer, ageing and chronic diseases (Djojosubroto et al., 2003; Rudolph et al., 1999; Satyanarayana et al., 2003).

Some specialized cells maintain their capacity to divide and regenerate their telomeres due to the action of an enzyme called telomerase. Telomerase is a reverse transcriptase that elongates telomeres in some tissues (i.e. germ and stem cells; Flores et al., 2006). Telomerase activity declines during late oogenesis and early preimplantation development, so maximum telomere length is thought to be established during early oogenesis (Wright et al., 1996).

The telomere theory of reproductive senescence supports the idea that telomere shortening in late exit from the fetal production line and a long interval before ovulation in the adult thus causes reproductive ageing in women (Blasco et al., 1999). For example, in null mice for the telomerase gene, as telomeres shorten across generations, oocyte and embryo development deteriorates (Hassold and Hunt, 2001).

Chromosomes frequently malsegregate during female meiosis leading to aneuploidy, failed implantation and miscarriage (Harper et al., 2004; Keefe et al., 2006; Munné et al., 1995). The mechanism by which telomere shortening causes this misalignment is not well understood (Blasco et al., 1999), but could result from improper pairing of homologous chromosomes and defective formation of synapses and bouquet formation during early meiosis (Rudolph et al., 1999; Siderakis and Tarsounas, 2007). Meiotic progression and chromosome alignment at the metaphase stage in oocytes of first-generation null mice for the telomerase gene were comparable to that of the oocytes of wild-type mice; however, fourth-generation null mice showed chromosome misalignment, disruption of meiotic spindles and very short telomeres (Liu et al., 2002).

In humans, telomere length of replicating somatic cells is inversely related to donor age (Vaziri et al., 1993), highly variable among donors of the same age (Slagboom et al., 1994), highly heritable (Jeanclos et al., 2000) and greater in women than in men (Benetos et al., 2001). In contrast to the considerable information known about telomere length in human extrauterine life, little is known about telomere length during embryonic development.

Experiments on the telomere length at different stages of bovine and mouse early development have shown that telomeres at the morula–blastocyst transition reset their length to a specific set point (Schaetzlein et al., 2004), supporting the theory of telomere restoration in mammalian preimplantation development. Xu and Yang (2000) demonstrated that telomerase activity is detected at fertilization in bovine embryos created by IVF and reaches its highest level at the blastocyst stage. Recent data show that telomere length is critically related to fragmentation in IVF embryos in humans but further studies are needed (Keefe and Parry, 2005).

This study measured the telomere length in embryos at 5 days post fertilization and assessed whether there is an association between the chromosomal complement of the embryo and its developmental rate using quantitative and conventional fluorescence in-situ hybridization (FISH). The aim of this study was to start unravelling the correlation between telomere length and chromosome aneuploidy in human preimplantation embryos, a relationship that has not been studied before in such detail.

Materials and methods

Experiments were performed in preimplantation embryos 5 days post fertilization from patients undergoing fertility treatment with chromosomal screening. Seven couples donated embryos that were unsuitable for transfer or cryopreservation after being diagnosed as abnormal by aneuploidy screening on the day 3 of embryo development. The data comprised 35 embryos consisting of 1130 blastomeres. Two control samples were used in each experiment along with the embryos: (i) maternal and paternal lymphocyte suspensions; and (ii) a human cell line of immortalized keratinocytes called HaCaT (German Cancer Research Centre, DKFZ, Heidelberg, Germany). The parental lymphocyte suspensions were also used to measure the efficiency of the quantitative and interphase FISH techniques. HaCaT cells were used as a positive control since they are modified so as to have standard telomere length of 4 kb (Boukamp et al., 1988). The HaCaT cell line allowed the conversion of the fluorescence intensity in each embryo cell to kilobases since fluorescence intensity has been found to be proportional to telomere length (Lansdorp, 1996).

Patient details and ethical consent

Patients who undergo chromosomal screening are referred for three main reasons: (i) those of advanced maternal age (AMA; females $\geq$40 years of age); (ii) couples with history of repeated miscarriage (RM, more than three times); and (iii) those with repeated failed IVF cycles (repeated implantation failure, RIF, more than three times). Research on all embryos donated to this study was carried out under licenses from the Human Fertilisation and Embryology Authority (HFEA) of the UK. Informed research consent was obtained from all couples. Approval was obtained by the Research Ethics Committee (REC3), North London, UK (reference no. 10/H0709/26, approved 8 June 2010).

Lymphocyte culture and counts

Lymphocyte culture from parental blood samples were carried out by standard methods and suspensions were prepared to be used in each experiment.

Embryo spreading

Embryos showing aneuploidy following a clinical preimplantation genetic screening (PGS) cycle and donated for research were spread on slides using the method previously described by Harper et al. (1994). The position of every blastomere was mapped so that it could be identified in each round of FISH. The method has been validated extensively and approved by the HFEA to be used as a diagnostic test for genetic screening. The overall efficiency (percentage of cells