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ARTICLE

Investigation of sperm telomere length as a potential marker of paternal genome integrity and semen quality

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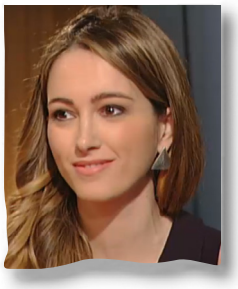

Federica Cariati obtained her Masters degree in Biotechnology of Reproduction Magna cum Laude from University of Teramo (2009), Masters in Medicine of Reproduction from University of Padua (2011) and her PhD in Biotechnology of Human Reproduction (2014) from the University of Rome "Sapienza", Italy. Since 2015, she has been resident in clinical pathology at the University of Naples "Federico II". Currently, she is working as a research assistant at CEINGE-Biotecnologie Avanzate, Naples. Dr Cariati's current research is on the effect of chromosome abnormalities on human reproduction. Her work also involves the development of new methods for sperm DNA analysis.

Abstract Recent studies have reported shorter sperm telomere length (STL) in men with idiopathic infertility. The aim of this study was to measure STL in semen samples from men to evaluate whether STL variation is associated with chromosomal abnormality, DNA fragmentation, traditional semen parameters, IVF outcome, or all four factors. A significant correlation between telomere length and diploidy was observed ($P = 0.037$). Additionally, STL was found to be positively associated with sperm count ($P = 0.006$); oligospermic samples had particularly short telomeres (0.9 ± 0.1 versus 1.4 ± 0.1; $P = 0.0019$). The results confirmed a link between sperm DNA fragmentation and aneuploidy, previously proposed ($P = 0.009$). A negative relationship was demonstrated between sperm concentration and aneuploidy and Sperm DNA framentation ($P = 0.03$, $P < 0.0001$, respectively). For a subset of 51 of the 73 sperm samples used for fertilization, IVF outcomes were known. A total of 17.6% of these samples had atypical STLs. None of these samples produced an ongoing pregnancy. In contrast, the pregnancy rate for samples that had STLs in the normal range was 35.7% ($P = 0.044$). In conclusion, STL has potential as a fast and inexpensive form of sperm quality assessment.

KEYWORDS: aneuploidy, human sperm, male infertility, sperm DNA damage, telomere length

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Introduction

Telomeres are structures composed of non-coding tandem repeats of a TTAGGG DNA sequence, located at the end of each chromosome arm (Moyzis et al., 1988). Together with numerous telomere-associated proteins, including the six-member Shelterin complex, telomeres play a key role in the maintenance of chromosome stability and genome integrity (O'Sullivan and Karlseder, 2010). Additionally, telomeres serve important functions in meiosis, helping to facilitate chromosomal alignment, pairing, synapsis and crossing over, which are critical steps during gamete formation (O'Sullivan and Karlseder, 2010).

Telomere length is maintained by telomerase, a reverse transcriptase, that is maximally expressed in a few types of highly proliferative cells, such as germ and neoplastic cells (Blackburn, 1991). In normal somatic cells, telomeres shorten with each mitotic division, eventually reaching a critical length associated with the induction of senescence, cell cycle arrest and apoptosis (Harley et al., 1990).

Three telomere-specific differences between somatic cells and sperm are presently known. First, in contrast to the telomeres of somatic cells, those in sperm do not shorten with age, ensuring the transmission of intact chromosomes over generations. Indeed, several studies have reported that increasing paternal age is actually associated with longer telomeres in sperm and in the leukocytes of offspring (Aston et al., 2012; Eisenberg et al., 2012; Ferlin et al., 2013; Kimura et al., 2008; Unryn et al., 2005). The length of telomeric DNA in human spermatoza is 10–20 kb, about twice the 5–10 kb typically observed in somatic cells (Kozik et al., 1998). Second, different telomere binding proteins have been isolated in spermatoza, and *in-vitro* studies have confirmed their involvement in telomere DNA recognition (Gineitis et al., 2000). Third, during spermatogenesis, telomeres migrate towards the nuclear membrane where they form telomere associations (Gineitis et al., 2000).

A relationship between telomere function and aspects of semen quality is an intriguing possibility, of potential clinical importance. Studies into the relationship between telomere length and different sperm parameters, however, have yielded contradictory data. In the case of sperm DNA fragmentation, a marker often assessed in fertility clinics to shed light on the genetic integrity of a sample, discordant results have prevented a consensus being reached on the association of telomere length and DNA damage (Moskovtsev et al., 2010; Thilagavathi et al., 2013a). It is conceivable that fragmentation, or other types of DNA damage, could result in delocalization of telomeres, with consequences for chromosome segregation and nuclear architecture. The arrangement of chromosomes within the nucleus has been proposed to be important for fertilization, appropriate gene expression, and early embryo development. Therefore, correct positioning of telomeres is likely to be of significance for viability (Ward and Zalensky, 1996).

A few studies have indicated that measurement of telomere length in somatic cells may provide useful information concerning reproductive potential. Shorter telomeres in the leukocytes of both men and women seem to be associated with some cases of idiopathic recurrent pregnancy loss (Thilagavathi et al., 2013b). At this time, however, little evidence supports the notion that variation in the length of sperm telomeres is related to reproductive capacity. Indirect data, suggesting that sperm telomere length (STL) may be important, comes from research showing that shorter telomeres can be detected in some samples from men with idiopathic infertility (Thilagavathi et al., 2013a). Few studies have been published on STL in relation to semen parameters, and the small amount of information that is available is contradictory. Thilagavathi et al. (2013a) did not find any correlation with conventional sperm parameters and telomere length, whereas other studies reported a positive association between STL and sperm count in young donors (aged 18–19 years) (Ferlin et al., 2013) and in infertile patients (Yang et al., 2015).

The biological reasons for variation in STL in samples from different men and its potential correlations with fertility, outcome of assisted reproductive treatment and other clinical features remain poorly understood. Moreover, no data have been reported on STL in relation to chromosome abnormality in human sperm. Therefore, the aim of this study was to provide a detailed assessment of STL in men at ages typically encountered at fertility centres, and to evaluate whether telomere length variation is associated with chromosomal abnormality, DNA fragmentation, traditional semen parameters, IVF outcome, or all four factors. Additionally, this paper provides information on the potential utility of STL assessment in the evaluation of male infertility, considering the extent to which the information can add to, complement, or replace that conveyed by other forms of semen assessment.

Material and methods

Participants

In this study, 73 samples from men aged between 31 and 52 years, who had requested sperm DNA fragmentation and aneuploidy analyses, were assessed. Participants were divided in normozoospermic (total sperm count ≥15 million/ml; $n = 54$) and oligozoospermic (sperm count <15 million/mL; $n = 19$). The study was approved in 2013 by the Institutional Ethics Committee of the University of Naples Federico II by protocol No. 42/13, and a written informed consent form was signed by all the participants involved in the study. The study was conducted in accordance with the principles expressed in the Declaration of Helsinki.

Sperm preparation

Standard semen analysis was carried out according to World Health Organization protocol (World Health Organization, 2010) after 2–4 days of sexual abstinence. An aliquot of semen sample was assessed using the Sperm Chromatin Dispersion Test (SCDt) for sperm DNA fragmentation (SDF) analysis, whereas another was used for the analysis of chromosome numerical abnormalities. Multi-colour fluorescence in-situ hybridization with probes specific to chromosomes 13, 18, 21, X and Y was used. This has previously been described (Enciso et al., 2013). Additionally, a maximum of 10×10^6/ml of sperm was centrifuged using Percoll gradient (PureSperm, Nidacon International AB, Sweden) and checked under the micro-