Telomeres in human oocytes and embryos: maternal contribution to chromosome (in)stability?

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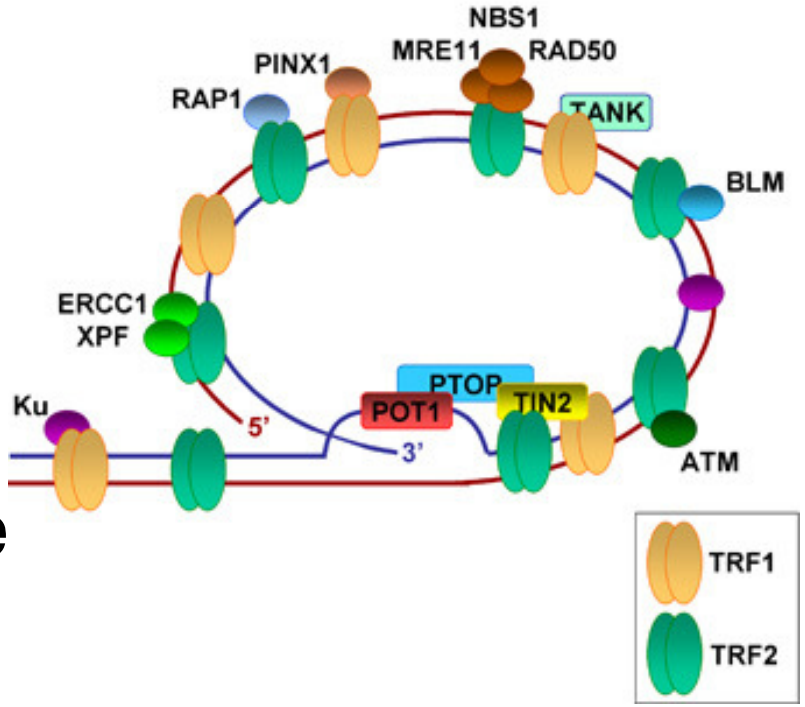
Outline

- Background
  - Telomere function
  - Shortening and extension mechanisms
- Telomeres in oocytes and sperm
- Fertilisation
- Telomeres in embryos
- New data on telomere lengths in human oocytes and embryos

Background

- Telomeres are repeated sequences of DNA at ends of chromosomes – TTAGGG –
- Specific conformation and surrounded by proteins that protect free ends from degradation

Abb. 1:
Struktur der Telomere mit stabilisierenden Proteinen

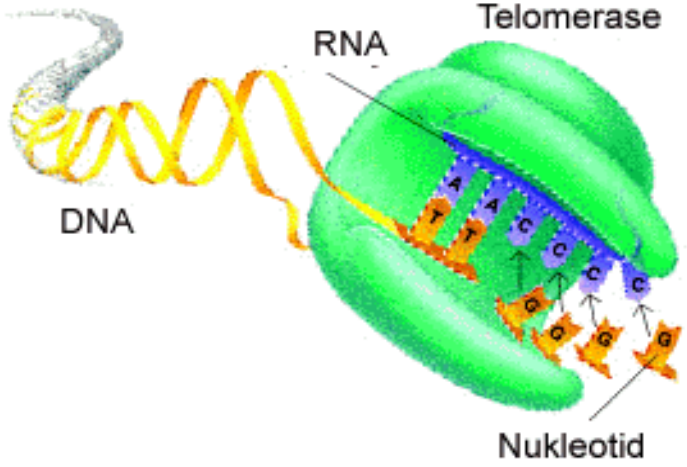


Background

- Functions:
  - ‘End replication problem’ – DNA polymerase cannot replicate the very ends of chromosomes, hence DNA shortens slightly with each replication. Telomeres avoid loss of critical coding DNA.
  - Control movement of chromosomes
    eg interacting with spindle,
    other chromosomes’ telomeres
    chromosome looping in spermatozoa

Background

- Telomeres shorten gradually with age due to DNA replication
- Stem cell telomeres shorten less than somatic
- Short telomeres recognised by DNA repair mechanisms due to inadequate protein cap
- Short telomeres promote end-to-end joining of affected chromosomes and chromosomal instability.
- Critically short telomeres cause cell senescence/apoptosis via p53
- **Telomerase** – reverse transcriptase enzyme, ribonucleoprotein, synthesizes TTAGGG repeats at chromosome ends resulting in gradual lengthening. Targets shortest telos in cell
- Telomerase present in stem cells, immortalised cells, most cancer cells



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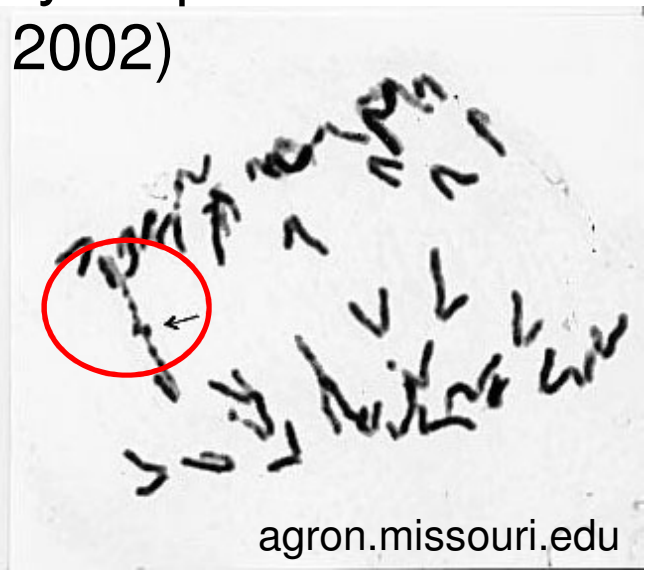
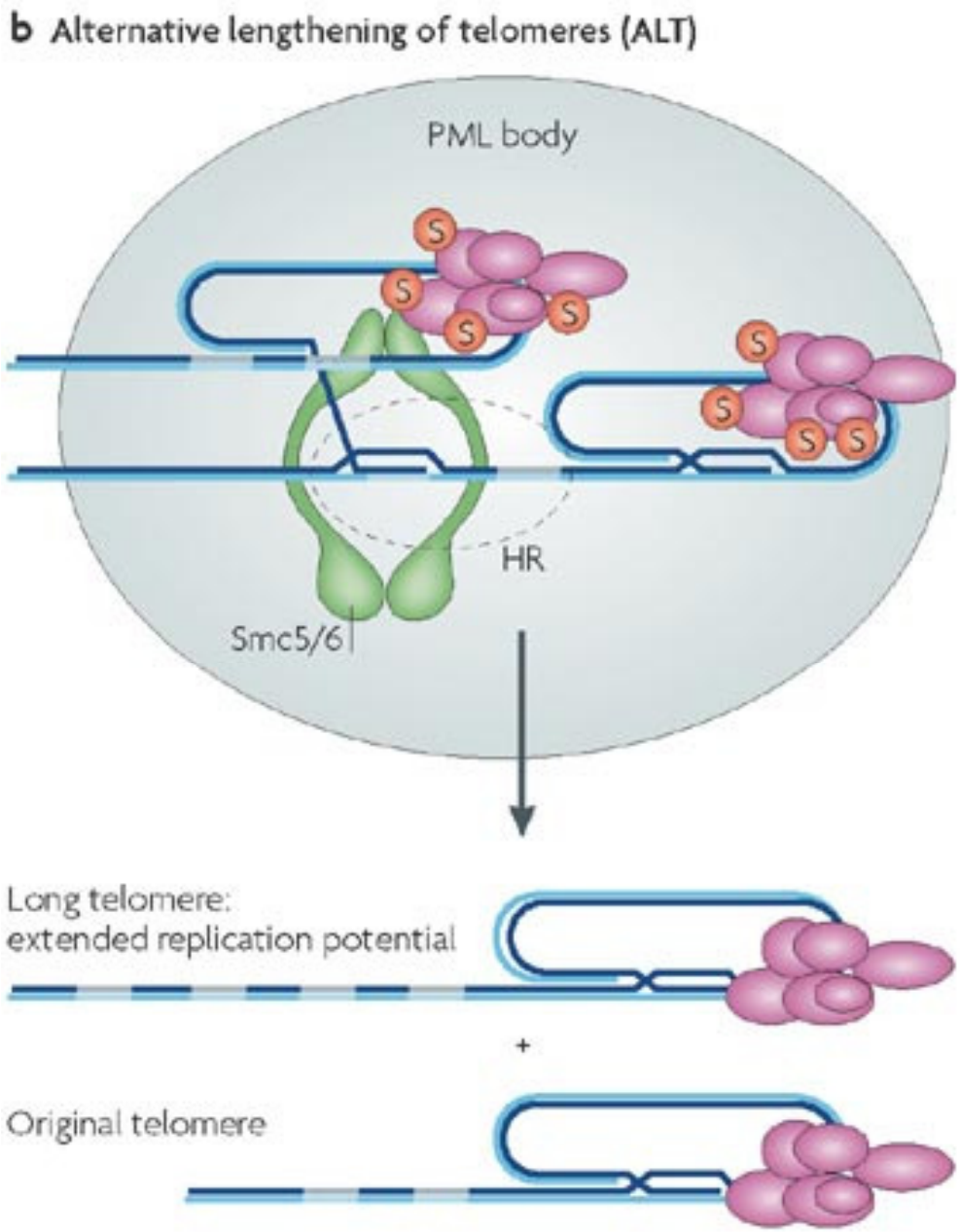
Background

- Major changes in telomere length occur at specific or random occasions.
  - Sporadic loss of telomere (exogenous DNA damage, problems with DNA repair, or spontaneous)
  - Sister chromatid fusion and anaphase bridging leading to 'break-fuse-break cycles' or possibly failure of chromatid separation (oocyte-specific mechanism in mice, Koehler et al, 2002)
  - Deletions at termini
  - Further chromosomal instability
- Irrespective of telomerase



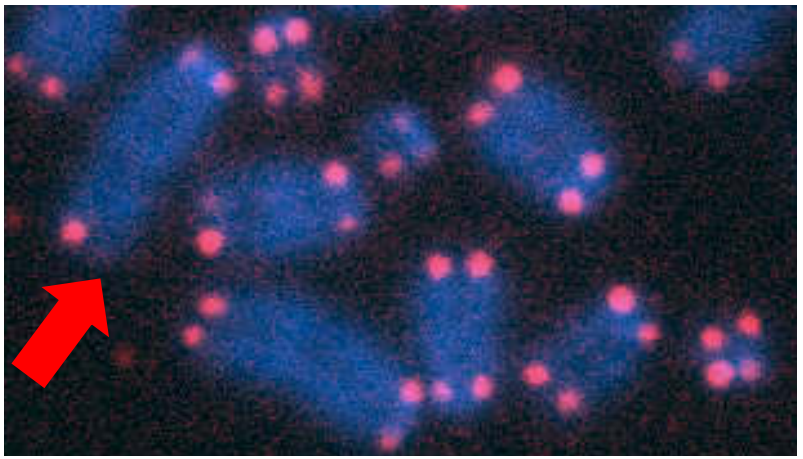
Background

- Quantum gains of telomere length due to
  - Telomere ‘healing’ = direct addition of telomere repeats to the ends of broken chromosomes
  - Non-reciprocal translocations – ie capture of the ends of other chromosomes (perpetuates chromosomal instability)
  - Duplication of the ends of chromosomes
  - ALT (‘alternative lengthening of telomeres’) by recombination, particularly between sister chromatids (SCE), leads to heterogeneous telomere lengths
- Mechanisms can coexist with telomerase



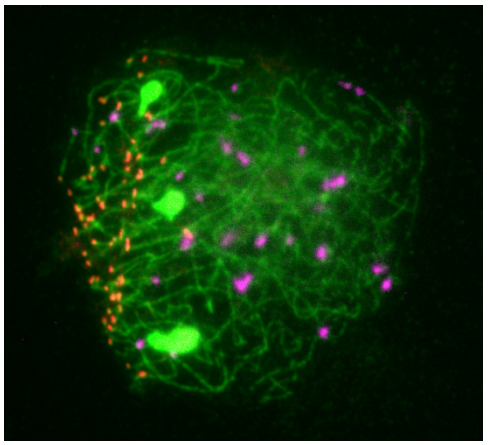
Murray and Carr, 2008 Nature Reviews | Molecular Cell Biology

- Alternative lengthening of telomeres pathway
- Increases variability in telomere length

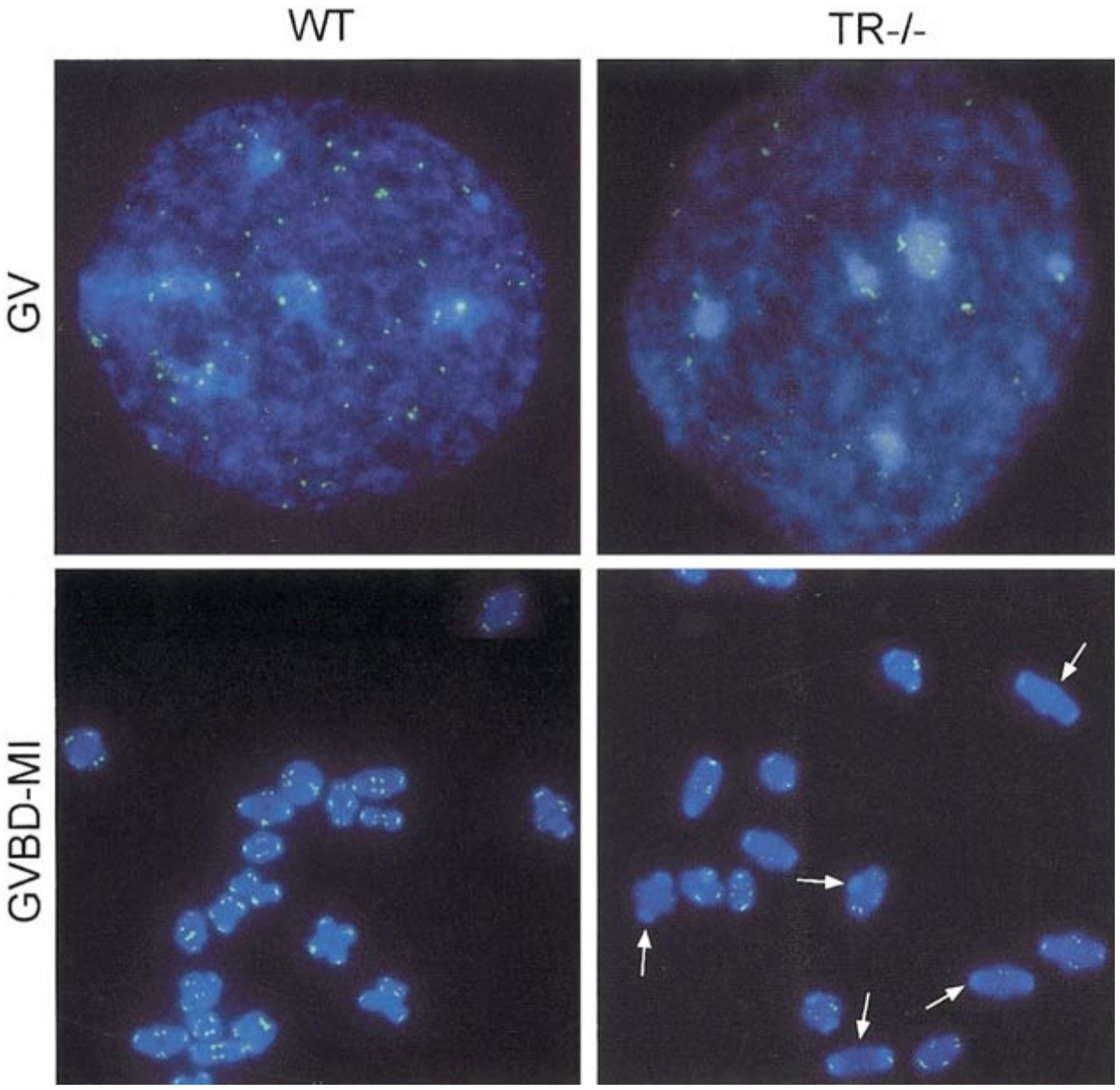


Sister Chromatid Exchance (SCE)

Telomeres in oocytes



- Telomeres control chromosome movement in prophase I (bouquet formation), for homologous pairing and interaction with microtubules eg spindle. Time lapse shows motility at key stages
- In telomerase-null mice, short telos in late generations associated with infertility, abnormal spindles and misalignment of metaphase chromosomes (Liu et al, 2004)
- Telomere length in human (unfert) oocytes correlated with embryo quality (fragmentation) in sibling fertilised oocytes and eventual pregnancy outcome (Keefe et al, 2007).
- Telomeres lengths of human oocytes found by Keefe et al were low (6-7kb).

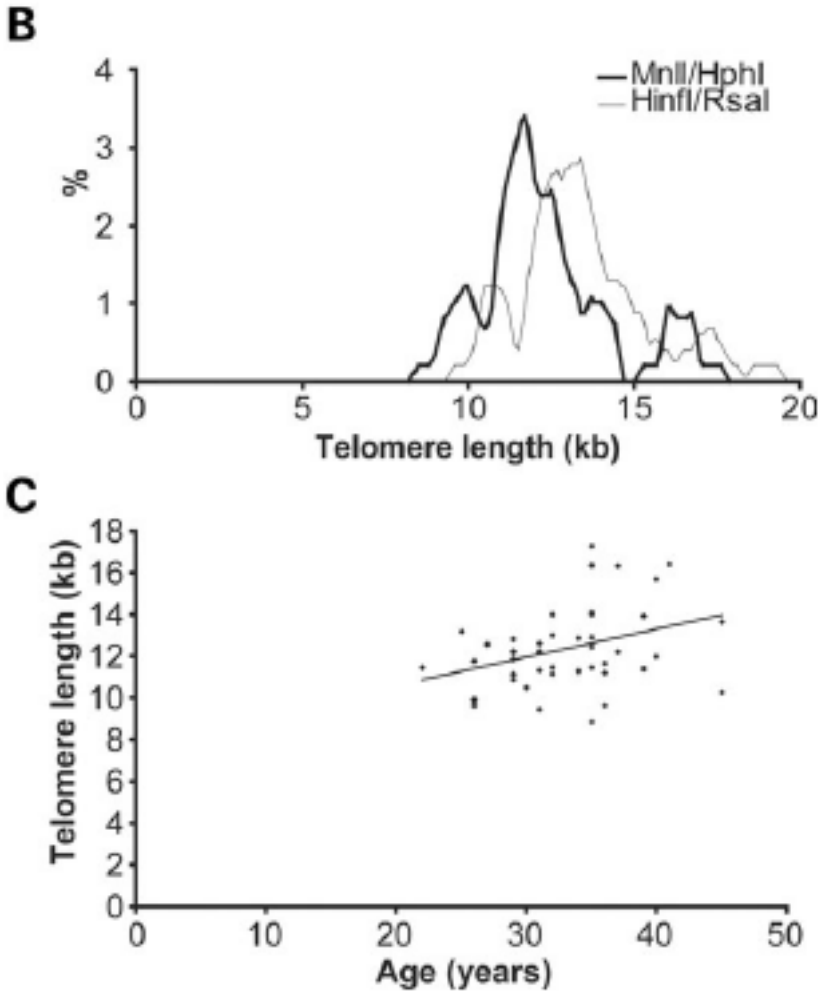


- Fewer spots seen in null than wt mice at GV

Liu et al, 2002

Telomeres in sperm

- Extensive variation in genome-wide telomere length (avg 12.5, range 8-17.5kb)
- Populations of sperm with short, medium and long telo lengths identified in individuals (Baird et al, 2005)
- High prevalence of substantial telomere truncations. Baird et al, (2005) estimate only 19% of human sperm have normal telos at all chromosomes
- Telomere length inversely proportional to telomerase as cells progress through male germ line (Achi et al, 2000)
- Critically short telomeres associated with sperm DNA fragmentation (Rodrigues et al, 2005)

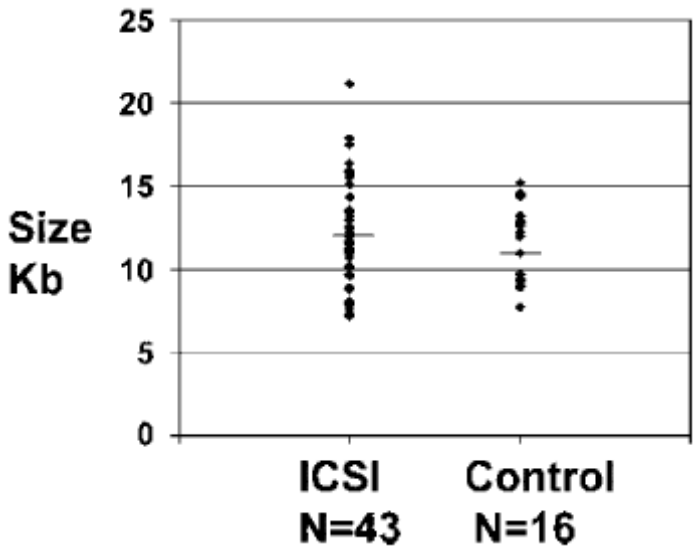


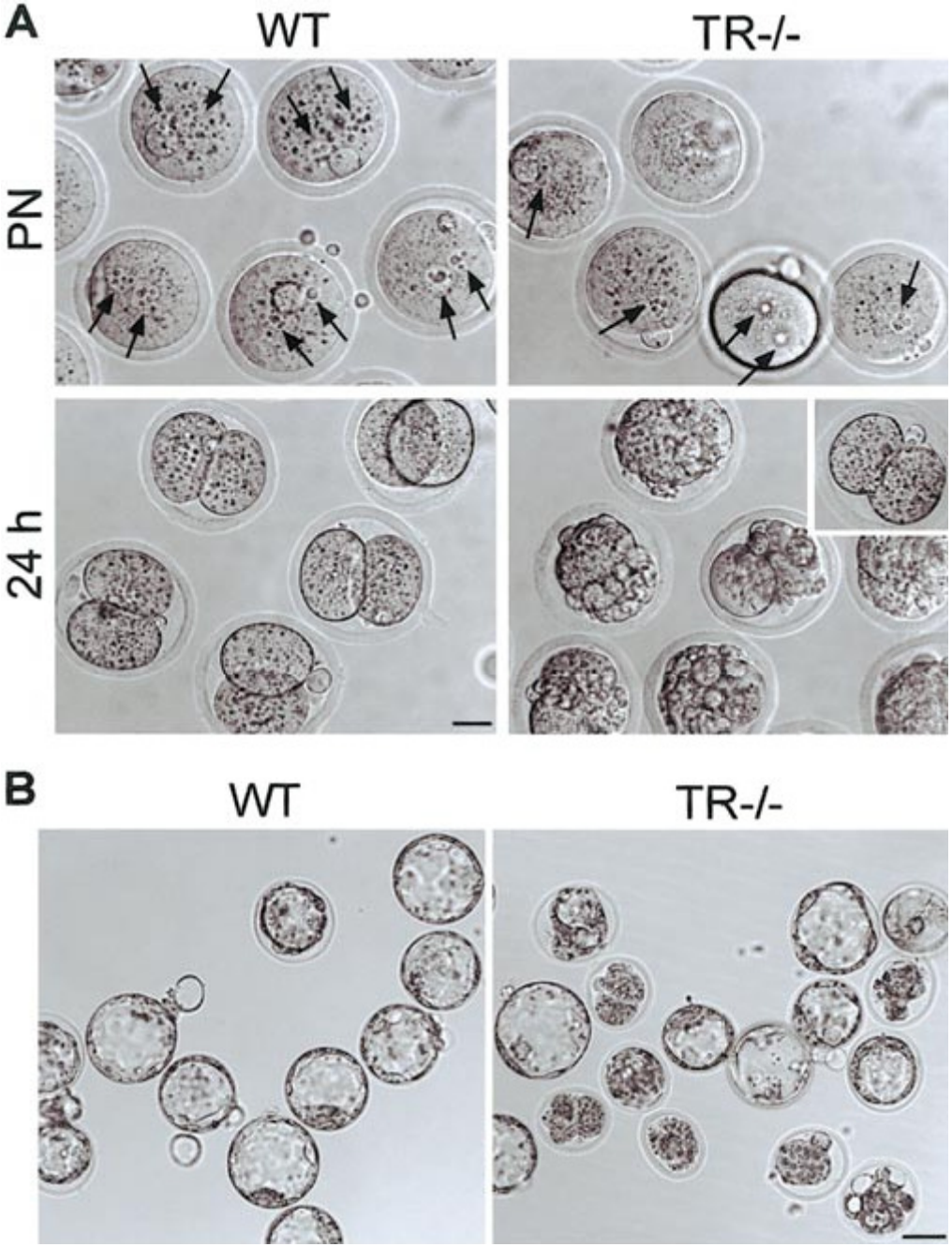
- Short, medium and long telomere length populations
- Sperm telomere length increases with age

Baird et al, 2005

Fertilisation

- Liu et al (2007) found oocyte telomere length short (surprisingly) and significant lengthening of telomeres between zygote and 2c (mouse)
- Telo length of parthenotes was greater than after fertilisation (mouse)
- In late generation telomerase null mice, both oocyte and sperm have similar contribution to loss of function (fert and cleavage) Liu et al 2002
- Newborn telomere lengths in ICSI vs controls aged 0-19 (Robinson et al 2005)





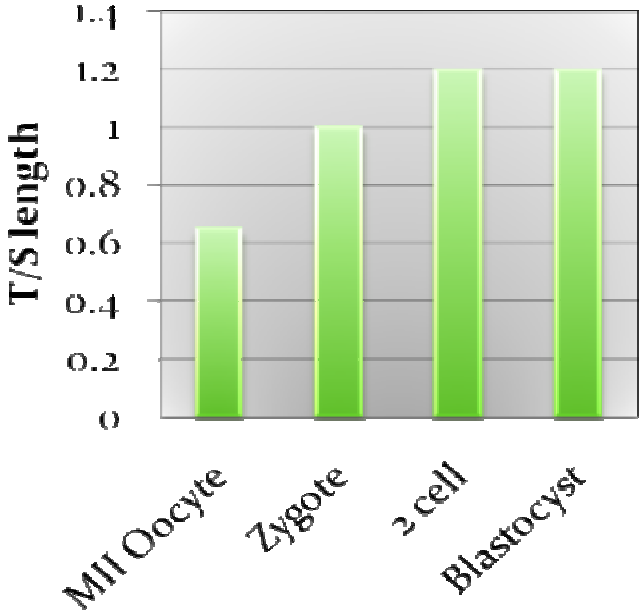
Liu et al, 2002

- Failures of fertilisation and embryo development in telomerase null mice

Telomere length in pre-implantation embryos

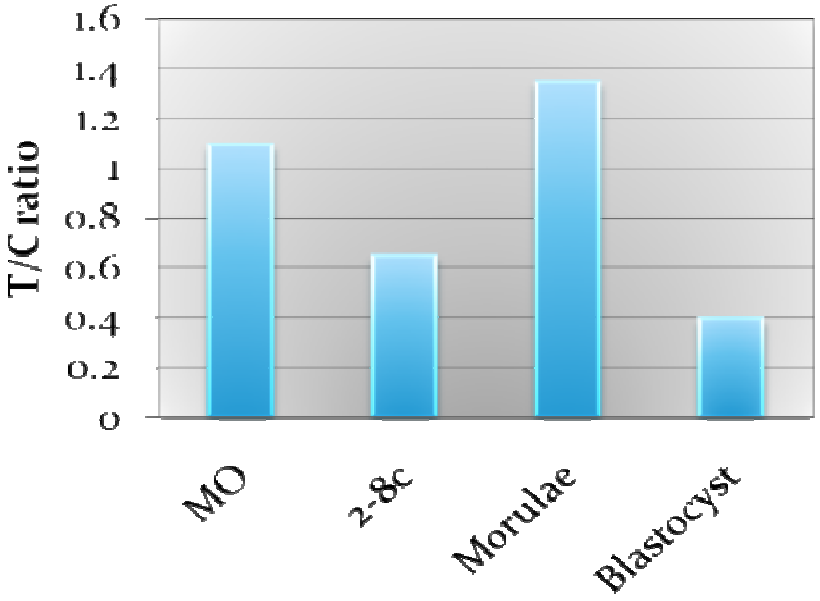
- Telomere lengths change during pre-implantation development (mice and cattle)

Mice



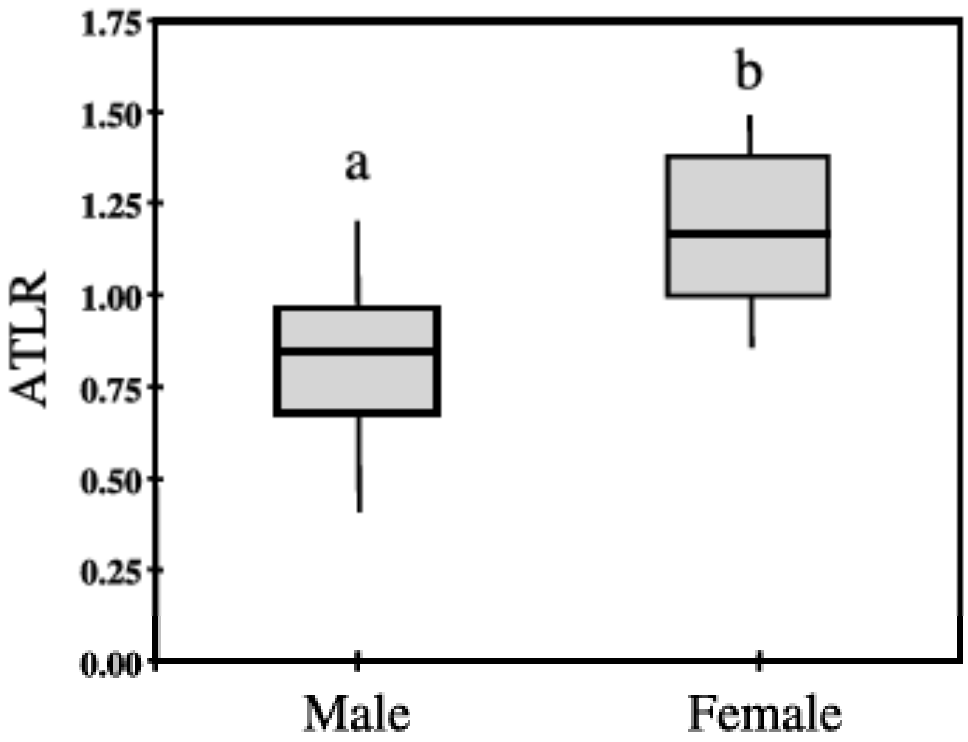
Liu,L,et al(2007) Nature Cell Biology 9 1436-1441

Cattle



Meerdo, L.N. et al (2005) Cloning and Stem Cells 7, 62-73

Bermejo-Alvarez et al 2007



P<0.01

- Average telomere length ratios longer in female than male bovine blastocysts
- Possible epigenetic regulation of telomere length or vv
- Possible sex-specific variability in e.g. resistance to oxidative stress.

- In telomerase null mice, telomere extension in early embryos mediated by ALT-SCE (sister chromatid exchange), but extension was greater in w/t mice, so telomerase may also be active. Liu et al (2007)
- Schaetzlein et al (2004) found an increase in telo length at blastocyst in mice that was entirely due to telomerase.
- In cloned embryos of cattle (4-5 kb, Lanza et al, 2000) and mice (Wakayama et al, 2000), telomeres of the donor cell are lengthened, depending on donor cell type.
- No data on telomere lengths in human embryos.

Hypothesis:

- that telomere length is important for, and may be a marker of, human pre-implantation embryo quality.
- that the oocyte's DNA damage repair mechanisms modify telomere lengths of incoming sperm

Methods

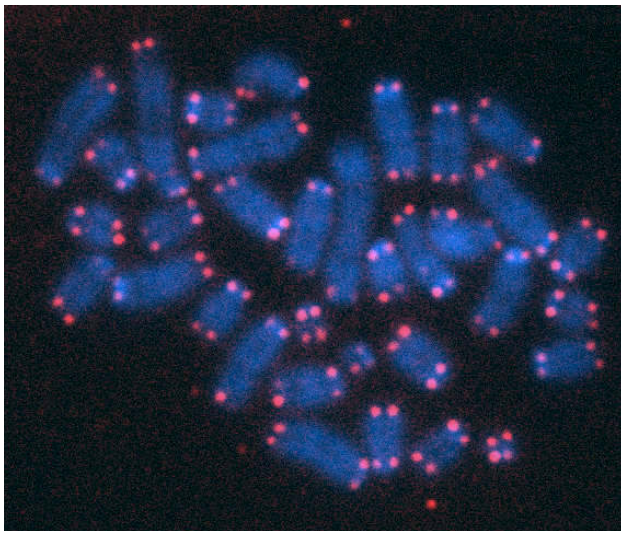
- Human embryos, donated to research (R0155)
  (Thawed), (cultured), zona removed
  Cells dissociated with Ca^{++}/Mg^{++} free medium
  Spread with citrate and Tween 20
  Fixed with methanol/acetic acid (Dozortsev et al, 2001)
- Control cells added to slide (mouse L-5178Y-S having known telomere length of 7kb)
- FISH using fluorescently labelled quantitative PNA probe for telomere sequence (DAKO). DAPI for chromatin. Olympus IX81. Imaged with fixed exposure time.
- Telomere length calculated using TFL-telo (Zijlmans et al, 1997) software, related to control telomere signals

Additional controls:

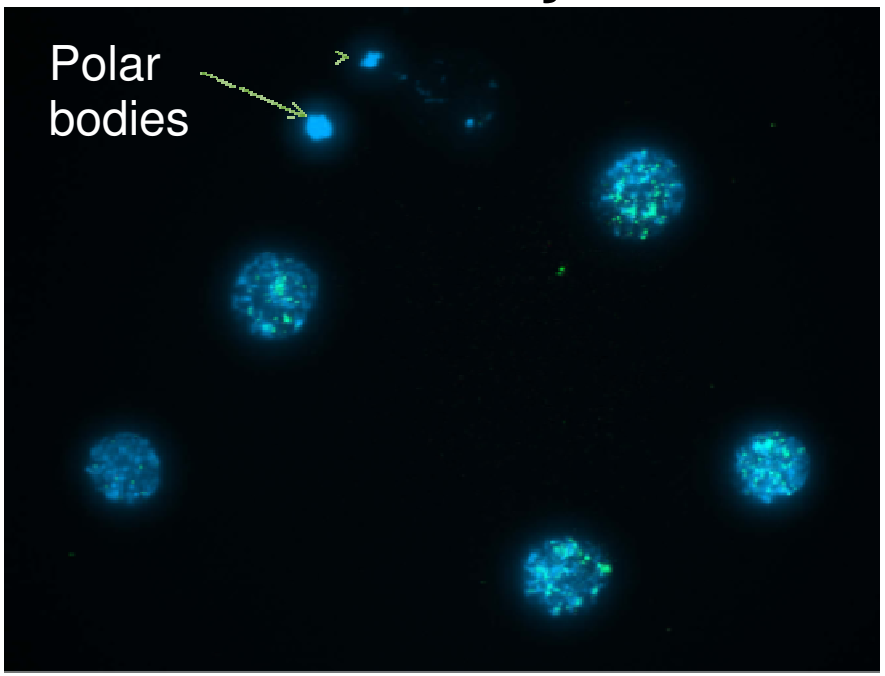
Condensed chromosomes – signal location at termini
Correlation of nuclear area with signal strength – no dilution effect

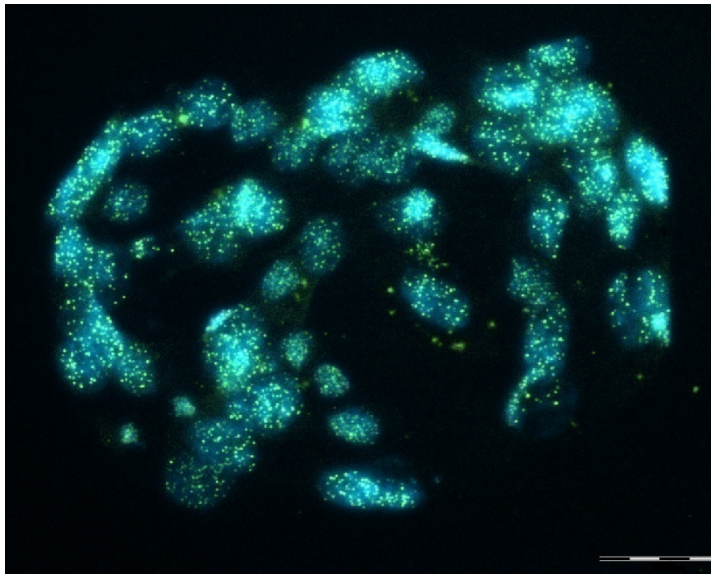
Telomere signals

Control



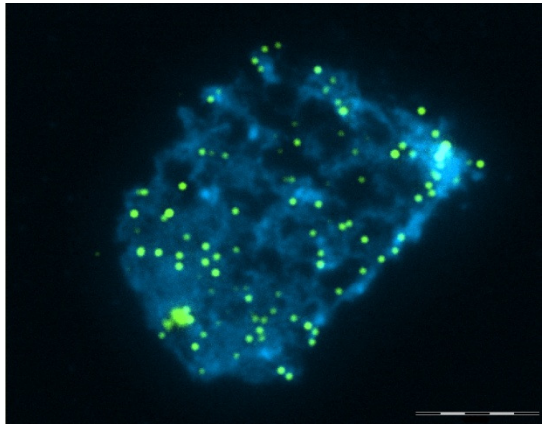
5-cell embryo





Blastocyst

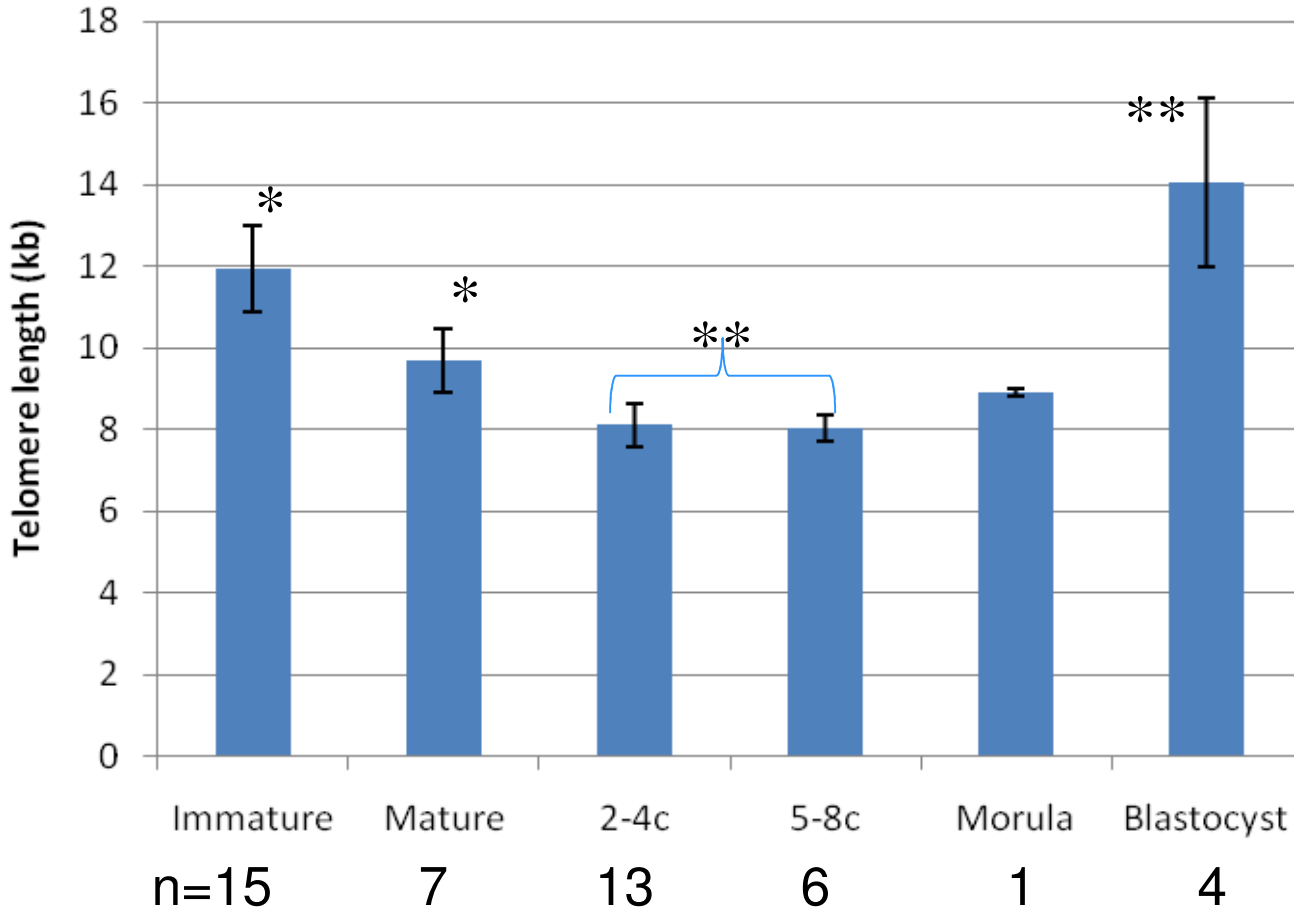
Blastomere



Average telomere lengths of oocytes (n=23 from 12 women) and embryos (n=24 from 9 couples)

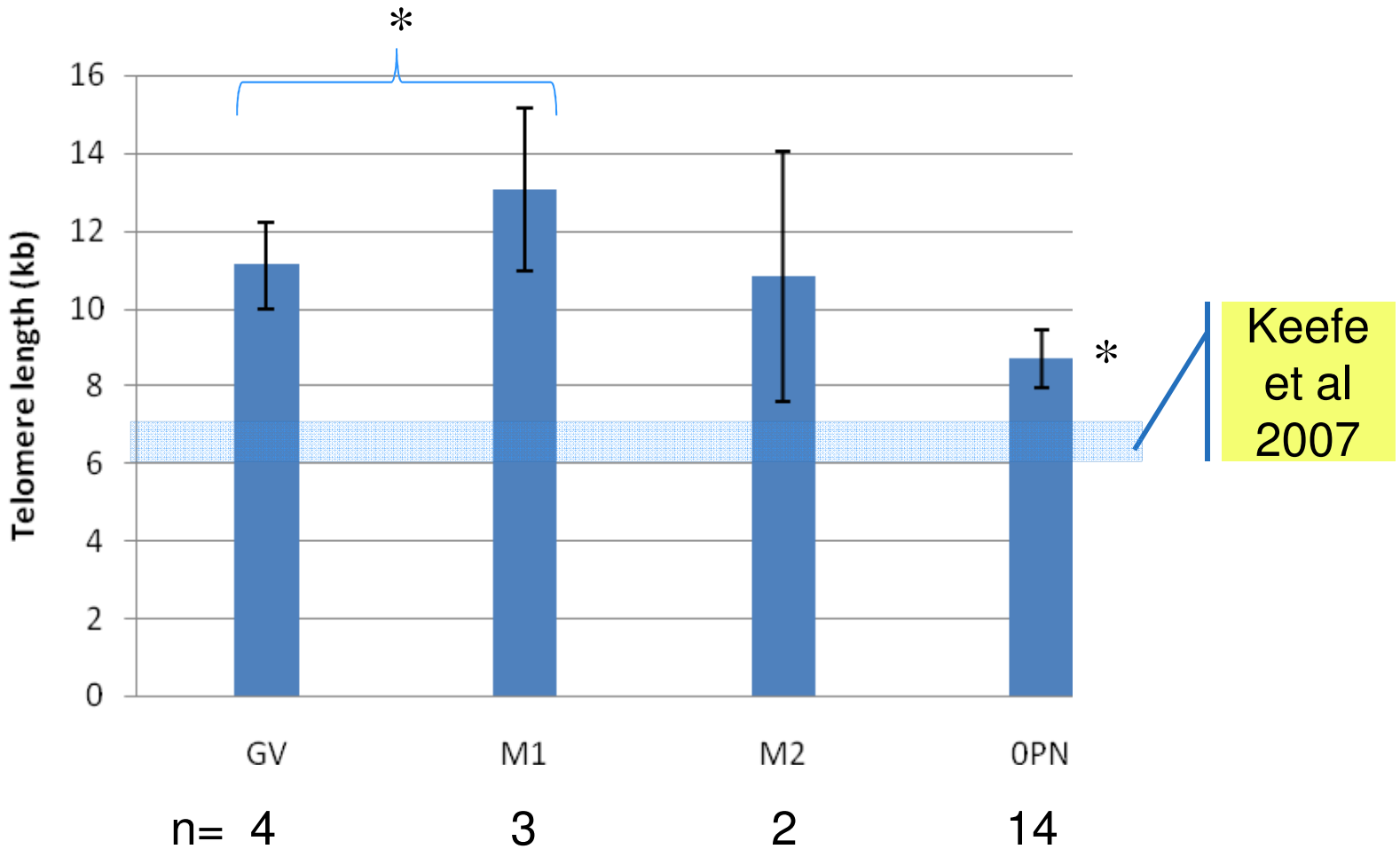
* p<0.05

** p<0.05

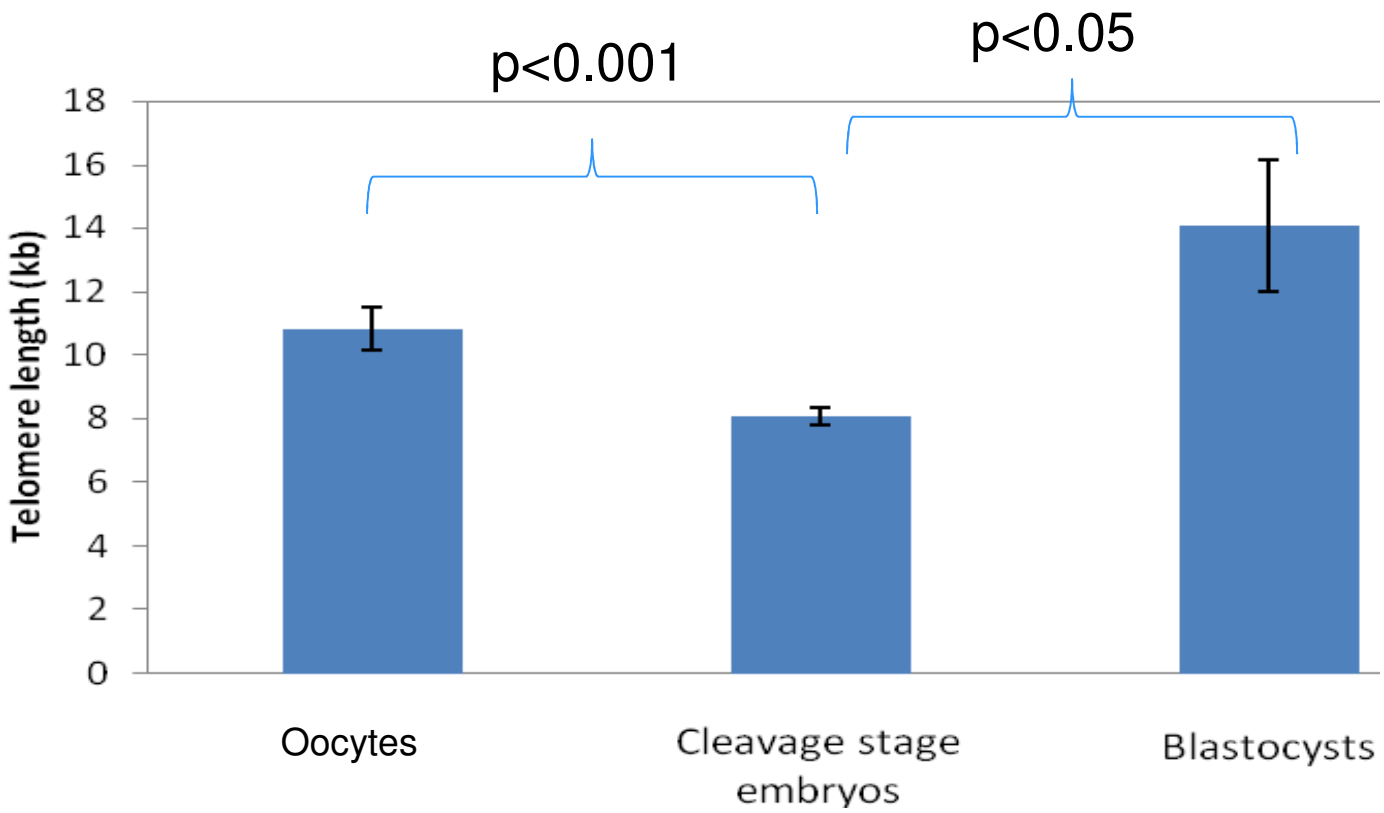


Average telomere lengths of oocytes at different stages of maturation

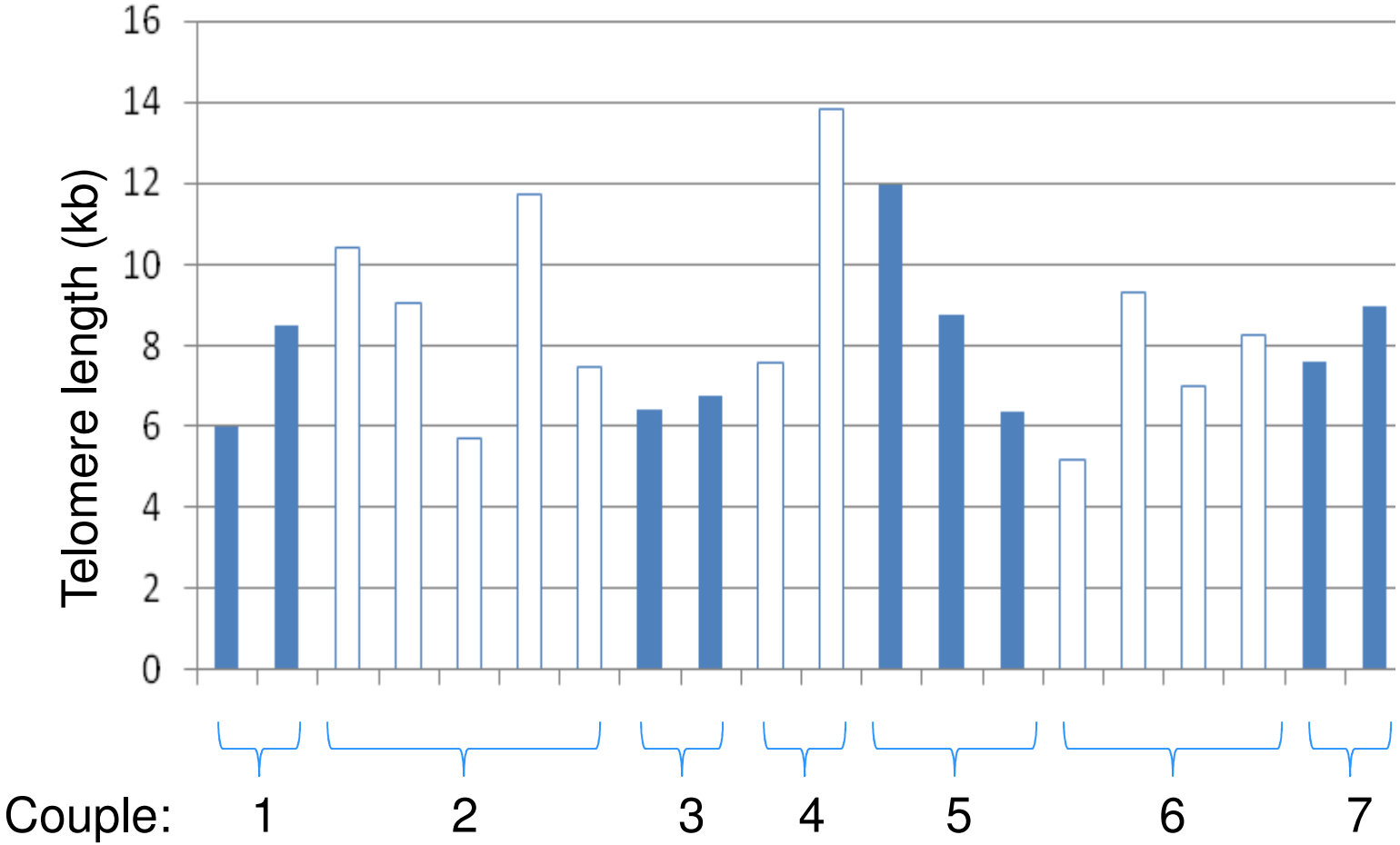
* p<0.05



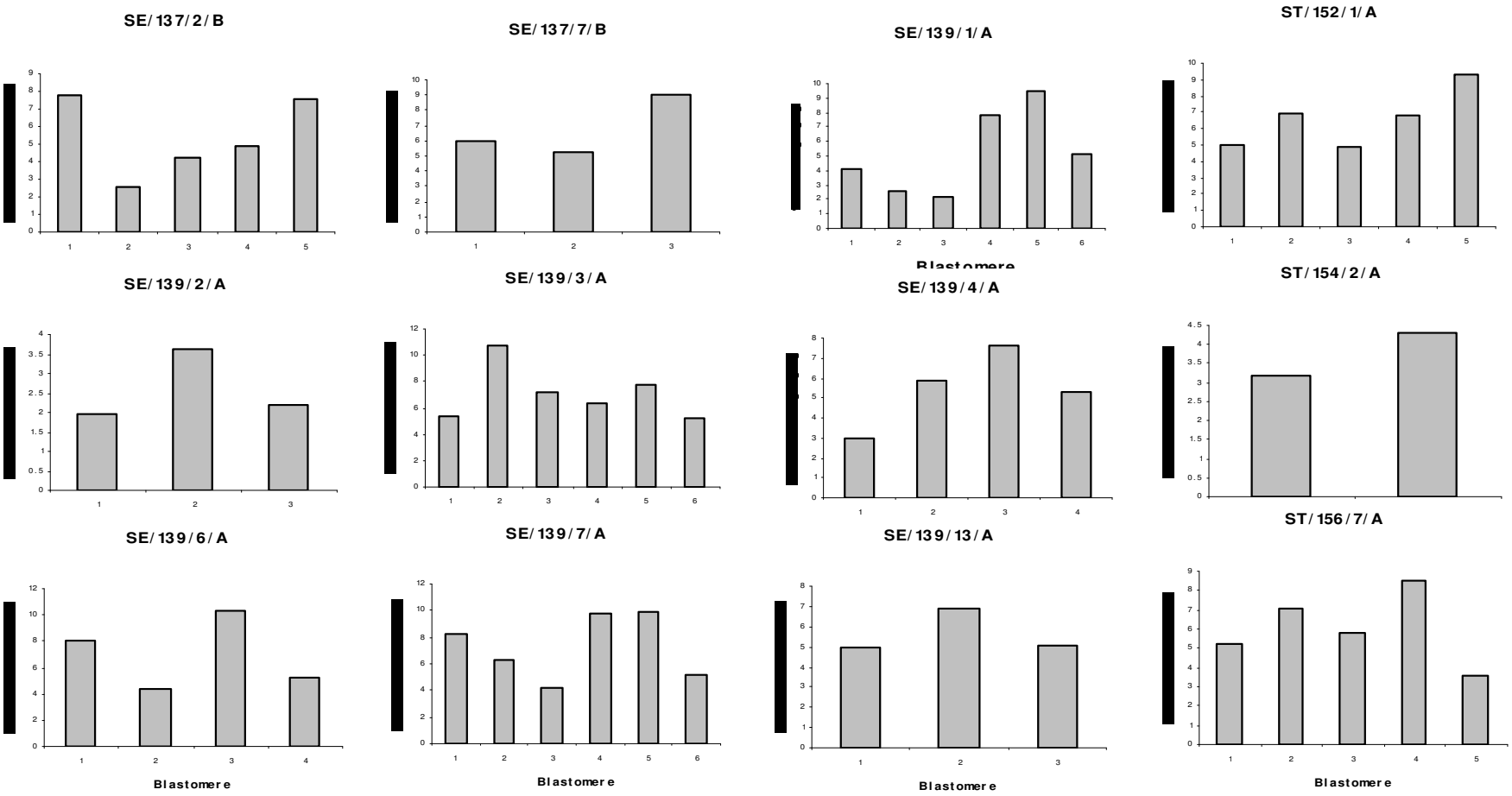
Telomere length is U-shaped during the pre-implantation period



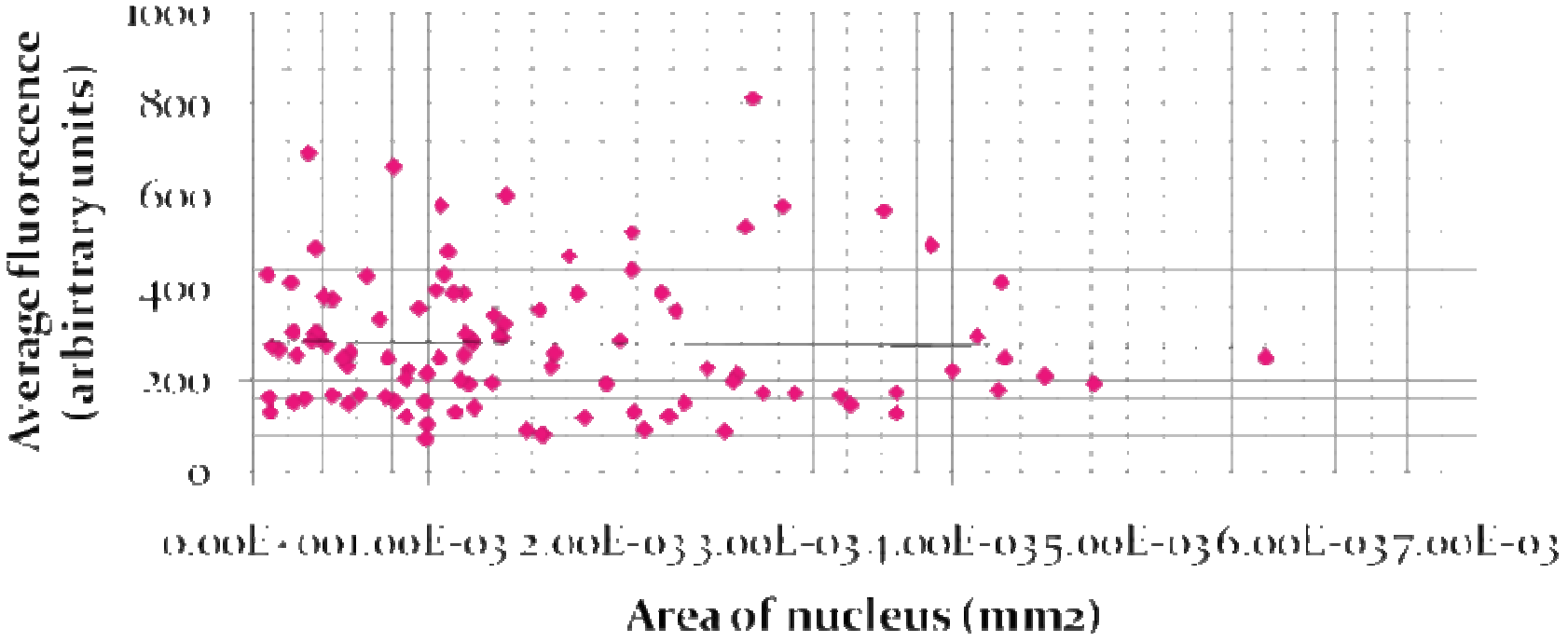
Variation of average telomere length among embryo cohorts of different patients



Intra-embryo variability

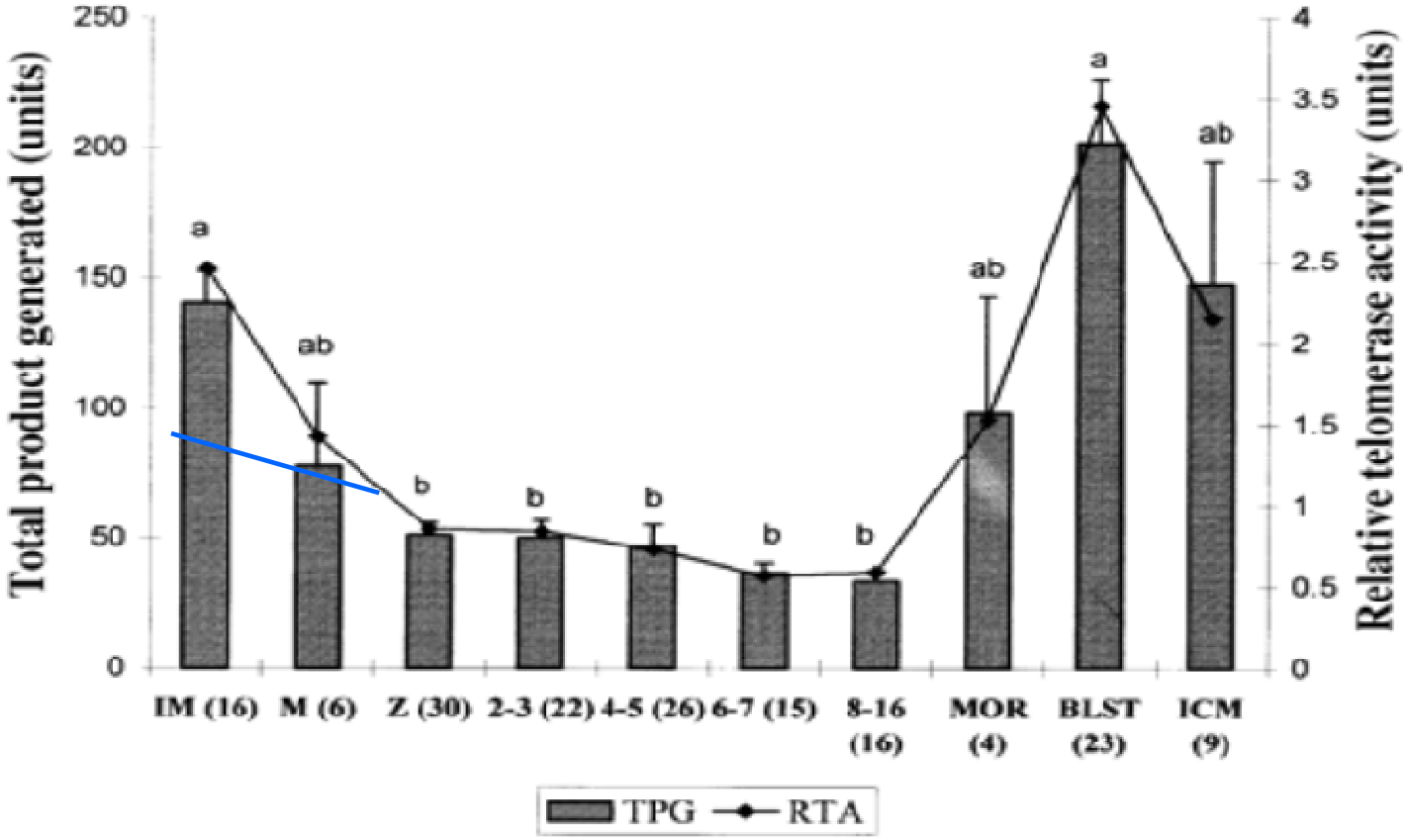


Comparison of measured fluorescence and nuclear area



- No correlation between fluorescence and area of nuclei. Differences in measured fluorescence are due to differences in telomere length alone

Telomerase activity in human embryos (Wright et al, 2001)



Conclusions

- Telomeres in human cleavage stage embryos are significantly shorter than oocyte telomeres ($p<0.001$)
- Telomere lengths at blastocyst are significantly longer than cleavage stage embryos ($p<0.05$)
- No difference between ongoing and arrested embryos
- No difference between frozen and fresh embryos

- *Propose: Telomere length of oocyte is likely important for ensuring sufficiency of ALT recombination-based mechanism in establishing embryonic telomere complement during cleavage phase, before telomerase becomes abundant at blastocyst.*
- ***Oocyte telomere length therefore influences embryonic genome stability through cleavage stages***

Questions arising

- How does telomere length change between oocyte and embryo?
- What affects telomere lengths in embryonic phase?
  - Assess mechanisms of telo extension and oxidative damage.
- What is the importance of inter-blastomere variation?
  - Impact of polarity, embryonic genome activation, mosaicism?
- More data needed.

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