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The deteriorating soma and the indispensable germline: gamete senescence and offspring fitness

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Running head: Germline senescence

18   **Abstract**

19   The idea that there is an impenetrable barrier that separates the germline and soma has shaped

20   much thinking in evolutionary biology and in many other disciplines. However, recent

21   research has revealed that the so-called 'Weismann Barrier' is leaky, and that information is

22   transferred from soma to germline. Moreover, the germline itself is now known to age, and to

23   be influenced by age-related deterioration of the soma that houses and protects it. This could

24   reduce the likelihood of successful reproduction by old individuals, but also lead to long-term

25   deleterious consequences for any offspring that they do produce (including a shortened

26   lifespan). Here we review the evidence from a diverse and multidisciplinary literature for

27   senescence in the germline and its consequences; we also examine the underlying

28   mechanisms responsible, emphasising changes in mutation rate, telomere loss and impaired

29   mitochondrial function in gametes. We consider the effect on life history evolution,

30   particularly reproductive scheduling and mate choice. Throughout, we draw attention to

31   unresolved issues, new questions to consider and areas where more research is needed. We

32   also highlight the need for a more comparative approach that would reveal the diversity of

33   processes that organisms have evolved to slow or halt age-related germline deterioration.

34

35   **Keywords** Oocyte, sperm, parental age, Lansing effect, ageing, maternal effect

36

Introduction

While a mechanism whereby offspring inherit beneficial traits from their parents is central to the theory of evolution by natural selection, robust scientific information on the processes of heredity was lacking when Darwin put forward his theory in 1859 [1]. Being apparently unaware of the pioneering work of Mendel on inheritance, Darwin later suggested that inheritance might occur via 'gemmules', tiny particles that circulate around the body and accumulate in the gonads, a developmental process he termed 'Pangenesis' [2]. Attempts to test this idea, notably by Galton, provided no support and it fell by the wayside [3]. Towards the end of the nineteenth century, August Weismann put forward his 'germ plasm' theory, based on the idea of continuity of the germline, its high level of protection, and its isolation from the somatic cells [4, 5]. In contrast to Darwin, he proposed that there was no transfer of genetic information between the soma and the germline, a separation which came to be termed the Weismann Barrier. This distinction between germline and soma became central to the neo-Darwinian evolutionary theories developed in the early twentieth century. It has also been central to key theories of the evolution of ageing in animals, such as the disposable soma theory [6], with the soma being seen as the vehicle that prioritises, protects, and preserves the integrity of germline, passing it on to future generations. The central argument is that, while the soma is allowed to degenerate with age, the germline is protected and damage to it should not be allowed to accumulate, either within the individual or from generation to generation.

However, we now know that Darwin's gemmule idea may not be entirely fanciful [3, 7], and that the Weismann Barrier is not so impenetrable as previously thought [8]: various potential carriers of epigenetic hereditary information from the soma to the germline have been identified, particularly those involving DNA methylation, chromatin modification, small RNAs and proteins that can influence gene expression, and extra-cellular vesicles that potentially move from the soma to the germline [7-12]. Investigating the transfer of epigenetic information across the generations by both sexes is a fast growing field of research. Moreover, while it appears that germline DNA is indeed afforded special protection [13], germline mutations do occur, since neither DNA replication nor repair are perfect processes and external insults can also inflict significant damage.

So to what extent is the germline imperfectly isolated from the age-related deterioration generally evident in the soma? Does the germline itself also age, and if so in

what way? Is this different in male and female germ cells? How does this affect the germline DNA and other hereditary processes? Is it also the case that the material passed via the cytoplasm of the oocyte is adversely influenced by the passage of time, both by deterioration in the oocyte itself and in the somatic tissue that exists to protect it? Does all of this have implications for the shaping of animal life histories?

These questions are the focus of this review. First we consider briefly whether there is evidence of a negative effect of parental age on offspring health and longevity, and the routes whereby such an effect of paternal and maternal age could occur. We then focus on the germline itself, examine the evidence that it can deteriorate as the soma ages, and review the mechanisms by which this occurs. We then consider what this means for relevant aspects of life history evolution, in particular the scheduling of reproduction and mate choice. Throughout, we highlight and discuss the most critical gaps in our current understanding.

1.   Negative effects of parental age on offspring longevity

One of the first studies to demonstrate parental age effects on offspring health and longevity was undertaken by Alexander Graham Bell, inventor of the telephone. Towards the end of his life he developed an interest in heredity (unfortunately combined with one in eugenics). Using data from the family tree of William Hyde, one of the early English settlers in Connecticut, USA, Bell showed in 1918 that children born to older mothers and fathers had reduced lifespans [14]. Jennings and Lynch followed up this idea experimentally by using parthenogenically reproducing rotifers *Proales sordida* [15]; their results also suggested (while not being statistically significant) that the offspring of old females do not live as long as those of young females. This was taken further by Albert Lansing, using clones of the rotifer *Philodina citrina*. In 1947 he showed, through selecting old animals as breeders, that the offspring of old parents had a reduced lifespan [16], an effect that has become known as the Lansing effect. Furthermore, by creating parthenogenic selection lines in which he continually used the offspring of old or young individuals as parents for the next generation, his experiments appeared to show that this adverse parental age effect became magnified over generations, leading to the relatively rapid extinction of the old breeder line. In contrast, there was no change in lifespan or viability in lines based on selecting offspring produced only by young individuals [16].

It is important to note that almost all recent studies of the Lansing effect only consider two generations (i.e. they test whether offspring of old parents have a shortened lifespan), and

101   so cannot test whether (or how) the effect is or is not cumulative over successive generations,

102   as suggested in Lansing's original experiments. A partial exception is a study showing a

103   cumulative negative effect of maternal age on offspring in *Drosophila*: the lowest proportion

104   of eggs that reached adulthood came from old mothers that also had old grandmothers [17].

105   The extent to which a parental age effect on offspring fitness persists beyond the F1

106   generation, and whether it is truly cumulative, is little known in other taxa. However, a

107   substantial body of evidence does exist to show that the age of the parents at reproduction can

108   reduce offspring longevity in the F1 generation. Early investigations of effects of parental age

109   on offspring in sexually reproducing species (mostly *Drosophila* spp.) gave inconsistent

110   results (see [18] for a critical appraisal of these early studies), but more recent studies have

111   frequently found a negative effect on offspring longevity in a wide range of species including

112   humans [19-23], other mammals [24, 25], birds [26-29], rotifers, crustaceans, numerous

113   insects, yeast and nematodes [30-32]. These include studies where animals were raised in

114   consistent and benign laboratory conditions, such that the shorter lifespan of offspring

115   appears to be due to faster ageing independent of environmental conditions (e.g. [24]). A

116   reduced reproductive performance in offspring of older parents has also been reported in

117   some cases [26, 27]; while this is much less frequently reported than effects on lifespan (and

118   may not always be apparent [25]), it should be noted that studies of lifetime reproductive

119   effects of parental age under natural conditions are very limited ([25] and references therein).

120   Both establishing and teasing apart the causes of effects of parental age on offspring

121   viability is not straightforward. In sexually reproducing animals, both maternal and paternal

122   age can potentially adversely affect the offspring; in practice however, it can be difficult to

123   tease apart the two since the age of the two parents is often correlated under natural

124   conditions. There are many different pre- and post-natal routes for such effects. However, it

125   is important to mention that there can be causes of a negative relationship between parental

126   age and offspring viability that do not involve ageing of the germline – or indeed any ageing

127   process at all. For instance, it is important to recognise that previous reproductive effort could

128   have effects independent of parental age [33]. Many of the studies to date, particularly in

129   long-lived species, are non-experimental and cross-sectional (i.e. comparing young *versus* old

130   members of the population at a given time) rather than longitudinal (comparing the same

131   parents when they are young vs when they are old), and thus differential survival of parental

132   phenotypes into old age could mask or enhance effects, as could cohort effects since in many

133   studies the capacity to compare aged individuals born in different years is limited [34].

134   Germline senescence is a wide-reaching, multidisciplinary topic. We restrict our
135   review to mechanisms related to the ageing of the germline in animals where there is a
136   separation of the germline and the soma. We also confine ourselves to sexually reproducing
137   animals (noting the current bias in the literature towards vertebrates), and consider effects
138   operating via both eggs and sperm. We now briefly describe relevant aspects of the
139   production and storage of the gametes before discussing the evidence that they deteriorate
140   with parental age, focussing in particular on age-related changes in levels of *de novo* DNA
141   mutation and aneuploidy, telomere length and mitochondrial function since these are key
142   factors that could give rise to both transmissible and cumulative negative effects on offspring
143   health and longevity.

2.   Production of the germline and gametes

145   In most metazoan animals, the germline resides in the eggs and sperm. The gametes arise
146   from specialised cells, the primordial germ cells (PGCs), which eventually become located in
147   the gonads during early development. These cells are not pluripotent, and, under natural
148   conditions, can only give rise to gametes [35]. While in all plants and some animal taxa (e.g.
149   tunicates, cnidarians, flatworms) the PGCs can be formed from somatic cells, in most
150   sexually reproducing animals PGCs arise very early in embryogenesis by one of two
151   methods, termed the induction and inheritance modes. The induction mode, typical of
152   mammals, is apparently the more prevalent mode and is thought to be the ancestral condition;
153   here PGC formation is induced by cell signalling pathways activated by the zygote genome
154   [36, 37]. Alternatively, in the inheritance mode, PGCs arise from specialised germplasm
155   already present in the oocyte cytoplasm. This contains specific proteins and RNAs needed for
156   PGC formation. The inheritance mode occurs in many taxa including birds and, interestingly,
157   in the non-mammalian species typically used in developmental studies such as *Drosophila*,
158   *Xenopus*, and zebrafish [36]; both modes are found in insects and amphibians. It has been
159   suggested that the rate of evolution within taxa might relate to the mode of PGC formation,
160   but this has been shown not to be the case [38]. It remains unclear what favours one or other
161   mode of germ cell formation. The inheritance mode potentially increases the opportunity for
162   factors influencing gene expression to be transmitted from parents, particularly the mother, to
163   the embryo [37]. Whether the mode of origin of PGCs has any bearing on the susceptibility
164   of the resulting gametes to age-related damage is unknown. The PGCs form at the blastoderm
165   stage in the inheritance mode, but shortly before gastrulation (i.e. slightly later) in the
166   induction mode [36]. This could potentially influence age-related effects on germ cells, since

167   the number of cell divisions before PGC formation is higher in the induction mode [37]. In

168   both modes, after their formation the PGCs migrate to the genital ridges where they start

169   colonising the gonads, undergo epigenetic re-programming involving the erasing and

170   resetting of maternal and paternal imprinting, differentiate into male and female gametes and

171   proliferate [35, 39].

172   Detailed knowledge of most of the processes of gamete formation is still relatively

173   limited and comes largely from studies of mice and humans [35] and to a lesser extent, birds

174   [40]. In the ovarian tissue of mammals and birds, the colonisation of the genital ridge leads to

175   rapid mitotic division, followed by substantial cell loss. The remaining cells become the

176   primary oocytes and enter Prophase 1 of meiosis, progressing as far as the diplotene stage, by

177   which point homologous chromosomes have aligned, the chiasmatic bridges that occur at

178   apparently random points between the chromosomes have already formed, and crossing over

179   has taken place [39]. The primary oocytes undergo meiotic arrest at this point. Meiosis

180   resumes just before ovulation, with each primary oocyte giving rise to one haploid cell and

181   three polar bodies. In long lived species, the resumption of meiosis in some oocytes can be

182   much later in life (up to 50 years in the case of humans). This long period of meiotic arrest

183   may require unique methods of DNA repair and replacement of proteins, and is a stage at

184   which significant age-related deterioration could occur.

185   In mammals and birds the full stock of oocytes is generally thought to be produced

186   before birth, and substantial loss again takes place in post-natal life - in humans it is

187   estimated that <0.1% will be shed as mature ova during a female's reproductive life [39].

188   This process of atresia is poorly understood, but could be a mechanism to remove defective

189   oocytes [30]. At menopause in women, the stock of oocytes is depleted, but there is little

190   evidence of oocyte depletion limiting reproductive life in other species. Moreover, the

191   reduced fertility in older females is not simply a consequence of having run out of oocytes,

192   since other aspects of the reproductive process (such as induction of hormonally driven

193   oestrous cycles) become increasingly less controlled with age [41, 42]. Interestingly, it has

194   recently been suggested that *de novo* oogenesis can take place in adult mammals, though the

195   evidence is somewhat contradictory and the subject hotly debated amongst developmental

196   biologists [43]. In some other taxa, there is reasonably good evidence that oogenesis occurs

197   into adult life; such *de novo* oogenesis has been found to continue beyond sexual maturity in

198   *Drosophila*, some teleost fishes, amphibians and possibly reptiles [39]. As an extreme

199   example, in the long-lived deep sea rockfish *(Sebastes alutus)*, histological work has shown

200   the maintenance of follicular pools in females over 60 years old, with no indication of

201   follicular senescence or atresia, so that egg production may continue throughout life (which

202   can be for up to 90 years) [44]. On the other hand, sharks and sturgeons, also very long-lived

203   species, appear to have limited oocyte stores [44].

204   Significant cell proliferation also occurs when the primordial germ cells arrive at the

205   genital ridge of a male embryo; at this point a proportion of the cells become undifferentiated

206   spermatogonia. They then generally undergo mitotic arrest and enter meiosis only after birth.

207   The developmental pathway leading from germ cells to mature sperm does not begin in

208   earnest until puberty. At this point the spermatogonia rapidly increase in number by mitotic

209   division. It is estimated that male and female germ lines have undergone a similar number of

210   mitotic divisions (ca 30-35) by puberty [45]. Thereafter however, the number of cell divisions

211   increases rapidly with age in males; sperm are produced as required, via a mitotic

212   proliferative phase followed by two meiotic divisions which give rise to four haploid cells.

213   Most of the cytoplasm is then ejected and the mature sperm develop. This proliferation

214   continues through the fertile life of the male [39]. In the amniotes (reptiles, birds and

215   mammals) substantial changes to the epigenome occur as sperm pass along the epididymis,

216   and this is a potential source of the intergenerational transfer of environmental effects via the

217   male germline [11, 12]. Age-related changes could be induced by the soma at this stage, but

218   this has been little studied.

3.   Evidence that the germline does deteriorate with age

220   While it used to be assumed that the germline was ageless, there is now increasing evidence

221   that the gametes of both sexes gradually deteriorate alongside (albeit at a slower rate than) the

222   soma [46-48]. Considering first the female germline, it is now known that oocyte quality

223   declines with the age of the female. This is appreciated in human IVF clinics: the egg donor's

224   age (and hence the age of the egg) is known to be more critical to outcomes than the

225   recipient's age [49, 50]. High reproductive rate can reduce female fecundity in species with

226   high levels of egg production [51]. Sperm have also been shown to change with male age,

227   during both the pre-meiotic and post-meiotic phases [52, 53]. Sperm ageing has led to the

228   evolution of many responses (by both sexes) to prevent its adverse effects; these include the

229   production of large amounts of sperm, dense ejaculates, sperm rejection (by *both* sexes) and

230   multiple mating by females, as well as the evolution of signals of a male's antioxidant status

231   (i.e. his potential defences against sperm ageing) [53, 54]. It should be noted that both sexes

232 can potentially store sperm, during which time it could deteriorate: in the male prior to

233 mating, and in the female after mating but prior to fertilisation of the eggs [52]. Such storage

234 effects have been shown in guppies *Poecilia reticulata*: prolonged sperm storage slows sperm

235 swimming speed, and results in offspring that are themselves less fertile [55]. Relatively little

236 is known about whether the shelf life of sperm varies with male age. Interestingly, in toads

237 *Bufo bufo* it appears that hibernation can slow the ageing of stored sperm [56], and it would

238 be interesting to know if this generally occurs when metabolic rate is lowered. In

239 mosquitofish *Gambusia holbrooki,* the swimming rate of a male's sperm after ejaculation

240 declines with his age, and additionally with his prior reproductive effort, again highlighting

241 the need to separate these two factors [33]. Sperm that has been stored by a female guppy

242 tends to lose out in competition for fertilizations with fresh sperm [57]; whether this is due to

243 its poorer swimming performance or to cryptic selection by the female is unknown. These

244 signs of ageing in guppy sperm occurred over periods of many weeks, but similar patterns are

245 seen over days in other taxa. Female birds fertilise and lay one egg every 1-2 days and may

246 store sperm over the period when the clutch is being produced; however, fertilisation rate and

247 embryo growth and survival were found to be impaired when female Black-legged kittiwakes

248 *Rissa tridactyla* used sperm stored for more than 7 days [58]. Given this relatively rapid

249 senescence of sperm, it is intriguing that social insect queens appear somehow able to

250 maintain the quality of their stored sperm for decades (e.g. queen ants may mate once when

251 young and then produce eggs for up to 30 years [59]). Relevant data from social insects on

252 gamete deterioration during storage, or with the age of the male or female producing them, is

253 surprisingly limited. While egg and embryo size have been found to decrease (and offspring

254 mortality in response to stress increases) with age in honey bee *Apis mellifera* queens [60], it

255 is not clear if this was due to the senescence of the queen, her eggs or her stored sperm.

256 **4.   Causes of germline deterioration**

257 Some mechanisms of ageing in somatic tissues do not appear, so far as is currently known, to

258 be relevant to the germline. For instance, transposable elements (TEs) become more

259 mobilized in somatic cells as the cells become older, and are thought to be a major cause of

260 the increasing instability of their genome. However, germ cells have protective mechanisms

261 that silence TEs, particularly the Piwi-piRNA pathway that is particularly active in germ cells

262 [61, 62]. The widespread suppression of TEs in germlines suggests that this could have been

263 a significant selection pressure promoting isolation between the soma and the germline. Other

264 hallmarks of somatic ageing [63] would appear to be more likely to also have germline

265 effects, i.e. DNA mutations, telomere attrition and mitochondrial dysfunction. Others such as
266 the loss of proteostasis may also be important: for example, the greater levels of oxidative
267 damage to proteins in the oocytes of older female *Drosophila* has been linked to reduced egg
268 viability [64]. However, relatively little is known of the magnitude or pervasiveness of these
269 effects. We have therefore concentrated on the three ageing mechanisms for which there is
270 evidence of occurrence in the germline.

271 **(a) Mutations in the germline DNA**

272 Is there evidence that mutations in the germline DNA increase with age and could contribute
273 to germline senescence? There are many stages at which germ cells of both sexes are
274 potentially susceptible to DNA damage. This can be due for example to replication errors,
275 faulty repair processes and chromosomal non-disjunction, environmental factors such as
276 chemical or thermally induced damage, or damage resulting from exposure to internally
277 generated Reactive Oxygen Species (ROS). Such damage can occur during both the
278 formation and storage of the gametes. Germ cells appear to have significantly superior
279 genome maintenance mechanisms compared with somatic cells, partly as a result of more
280 efficient base excision repair systems [13]. However unrepaired mutations, while rare, do
281 occur and can increase with parental age in both sexes [52, 65-67]. Robust estimates of
282 germline mutation rates are still limited, but overall they appear to occur at a higher rate in
283 humans and other primates than in the other vertebrate and invertebrate taxa that have been
284 studied [68-71]. Most detailed information comes from studies of humans and mice. Point
285 mutations in germ cells are much more common in sperm than in oocytes and increase
286 significantly with paternal age: in humans a man is expected to transmit *ca* 40 mutations to a
287 child he fathers when he is aged 20, but twice this number when he is 40 [13, 72]. Note that
288 these estimates of mutation load include those in non-coding regions of the genome; a recent
289 study based on RNA-seq (so only considering coding regions) found no difference in
290 mutation frequency in sperm from old versus young male mice [24], and the importance of
291 any difference in mutation load in the non-coding regions is not currently known.

292 It is estimated that more than three-quarters of those human germline mutations that
293 do occur are paternal in origin, and the number increases with paternal age [45, 71, 73]. The
294 greater incidence of point mutations in sperm than oocytes has generally been thought to be
295 primarily related to the high rate of cell division of the male germ cells, and to the high
296 metabolic activity and limited repair of DNA in mature spermatozoa [13]. However, this

297 explanation has recently been challenged, since the difference between the incidence of
298 maternally- versus paternally-derived germline mutations is already evident in the offspring
299 of young human parents, and this difference remains relatively stable with increased parental
300 age [45]. Chromosome-based abnormalities such as aneuploidy are more commonly of
301 maternal than paternal origin [67], due to problems associated with the resumption of meiosis
302 in primary oocytes after a long period of arrest [67]. Aneuploidy increases sharply in humans
303 with maternal age, thought to be due to the maintenance of the chiasmata and sister chromatid
304 cohesion becoming less secure in older oocytes, with eventual failure of chromosomes to
305 segregate [74]. There is little evidence of increasing aneuploidy in the sperm of older men
306 [75]. In fact, the incidence of Down's syndrome is greatest in the children of very young
307 fathers once maternal age is taken into account [76]. It has also been suggested that the DNA
308 repair and replication capacity deteriorates with maternal age [45].While there are extensive
309 quality control processes that eliminate defective gametes in both ovaries and testes, the
310 efficacy of such processes may also deteriorate with parental age [52]. Germline maintenance
311 is likely to be very expensive, and the capacity to invest in this may decrease with age [77].
312 Interestingly, some repair of the genome is known to occur in the zygote after fertilisation,
313 and it appears that the oocyte is responsible for this repair to both maternally- and paternally-
314 derived DNA [67].

315 All of these sources of damage to the germline DNA can lead to reduced fertility in
316 older parents (because fewer gametes are undamaged), but also to an increase in defective
317 offspring where such gametes and zygotes escape the quality control processes. Little is
318 known about mutations in oocytes in species where oogenesis occurs throughout life, or of
319 how germline mutations are influenced by the age of reproduction in semelparous species, in
320 which maturation in some species can take many years – clearly potentially fruitful areas of
321 future research.

322 **(b) Telomere attrition in the germline**

323 Telomere attrition is thought to be an important factor associated with somatic ageing in
324 many, albeit not all, taxa studied in the laboratory and in the wild [63, 78, 79]. A key question
325 therefore is whether telomere loss occurs in germ cell DNA. Most of what we know about
326 telomeres comes from somatic cell studies. Telomeres are complex structures comprising a
327 variable number of tandem repeats of a DNA sequence (TTAGGG in most eukaryotes),
328 shelterin proteins and telomere repeat-containing RNAs. Telomeres cap the ends of the linear

329 chromosomes of eukaryotes, distinguishing true ends from double stranded chromosomal

330 breaks and preventing the triggering of a DNA damage response, thereby ensuring genome

331 stability [80]. They play a crucial role during cell division. Since the process of DNA

332 replication is incomplete at the end of the lagging DNA strand (the 'end-replication

333 problem'), the sequence loss is absorbed by the telomere and the protein-coding sequences

334 preserved. Telomeres are also involved in other aspects of cell division, the movement,

335 localisation and anchoring of the chromosomes to the nuclear envelope, the pairing of

336 homologous chromosomes and synapsis formation [81]. In addition to the end-replication

337 problem, increased telomere loss can also arise as a consequence of damage to DNA, for

338 example by ROS and other factors [82]. In the absence of restoration, telomeres therefore

339 become progressively shorter with each round of cell division and this important change in

340 the nuclear DNA of cells eventually has substantial consequences. Once the telomeres

341 become critically short, the genome becomes unstable; the cell enters cell cycle arrest

342 followed either by apoptosis or an altered, pro-inflammatory secretory profile. Unrestored

343 telomere loss therefore sets a finite limit on the replicative potential of cells. Progressive

344 telomere loss contributes to the deterioration of the soma with age [63] and in some species

345 telomere length or loss rates have been shown to be predictive of eventual lifespan [83-85]. It

346 has also recently been shown that experimental elongation of telomeres in mice results in

347 slower metabolic ageing and increased longevity, providing confirmatory evidence of the

348 causative role of telomere length in contributing to somatic deterioration in later life [79].

349   Telomeres can be restored by the reverse transcriptase telomerase, or by

350 recombination-based processes termed Alternative Telomere Lengthening (ALT). While the

351 basic mechanisms of telomere biology are highly conserved, the pattern of telomere loss and

352 restoration varies among species, individuals and tissues, in part in relation to the risk of

353 tumour formation associated with a requirement for large numbers of cell divisions; it

354 appears that, in mammals, broadly speaking telomere length positively covaries with lifespan,

355 while somatic telomerase activity negatively covaries with body size [86]. In many large

356 bodied and long-lived species, little telomere restoration occurs in the majority of somatic

357 tissues [87, 88]. Interestingly, it also appears that for some age-related diseases such as

358 atherosclerosis, shorter telomeres may be advantageous so further trade-offs may be involved

359 [89]. The pattern of telomere inheritance is difficult to measure, and different studies have

360 alternatively suggested a mainly paternal, mainly maternal, or no parental effect on offspring

361 telomeres [e.g. see 90, 91-94]. However, in many of these telomere inheritance studies

362 important confounding variables have not been taken into account and sample size is often

363 insufficient for results to be conclusive [95]. Much further work is needed.

364 Telomere maintenance is essential during development and gametogenesis and is

365 closely regulated, with telomerase and ALT being important at different stages [96, 97].

366 There has been a great deal of interest in the extent to which this close regulation might break

367 down in older parents, in whether shortened gamete telomeres might contribute to declining

368 fertility and embryo developmental problems at older ages, and whether short telomeres

369 might be transmitted across generations. To date, telomere length in germ cells has been

370 studied mainly in rodents and humans. While at one time it was thought that germ cells do

371 not show telomere attrition, it is now known that this is not the case, at least for mammalian

372 oocytes, where telomere attrition appears to play a central role in oocyte ageing [98]. In mice

373 and humans, oocytes have shorter telomere lengths than sperm, and oocyte telomeres are

374 amongst the shortest in the body, while those of sperm are amongst the longest [91, 96, 97,

375 99]. This is consistent with levels of telomerase being low in oocytes but high in

376 spermatogonia, although the generality of these patterns is unknown [92, 99].

377 During the meiotic arrest, the oocytes do not divide, but their precursor cells have

378 divided extensively. Therefore there is the potential for substantial telomere attrition during

379 the highly prolific mitotic stage following the primordial germ cells populating the

380 developing ovary. The oocytes from older women have weakened DNA and protein repair

381 mechanisms and impaired ROS metabolism [100], which, when combined with impaired

382 telomere maintenance (lower expression of the telomerase TERT gene and lower levels of

383 telomerase), leads to shorter telomeres in the oocytes of old females compared to those from

384 younger females [98] (Figure 1). Shorter telomeres have been found in the oocytes from old

385 compared with young mice [101], and are associated with lowered levels of the protein

386 SIRT6; experimental overexpression of SIRT6 promotes telomere elongation at the 2-cell

387 blastomere stage, suggesting that this protein is directly involved in the age-related decline in

388 telomere length in oocytes [102]. Oocytes with shorter telomeres develop abnormal meiotic

389 spindles and decreased chiasmata, which is thought to contribute to declining fertility with

390 age in human females [97]. Interestingly, there is evidence that the last oocytes ovulated by

391 older women come from those oogonia produced towards the end of the mitotic proliferative

392 stage [103]; having arisen from more cycles of cell division, they potentially have shorter

393 telomeres [97]. Additionally, oocyte telomeres might be damaged by ROS during the long

394 arrest prior to ovulation. However, it is very difficult to study oocyte telomeres in viviparous

395   species, and there is scope for much more work on oviparous species, especially those with

396   external fertilisation which offer the opportunity to examine telomere dynamics in both

397   unfertilised and fertilised eggs.

398   Sperm telomeres are obviously much easier to study than those in eggs. The continued

399   proliferation of cells to generate sperm throughout adult life may explain the presence of high

400   levels of telomerase in order to maintain sperm telomere length [81]. Again most data come

401   from mammals, where the picture with respect to age-related changes is mixed. For example,

402   sperm telomeres have been reported to both decline [91] and increase [101] with male age in

403   mice *Mus musculus*, but decrease in rats *Rattus norvegicus* [104]. In humans, telomere

404   lengths in sperm have been found to increase with male age [92, 105] in contrast to the

405   pattern in somatic tissues [106]. How an increase with male age occurs remains unclear

406   [106]. It could be due to expression of telomerase, or stringent quality control of sperm that

407   results in the removal of an increasing proportion of sperm with short telomeres as males age,

408   or be a consequence of cohort effects or differential survival of male phenotypes [106]. Loss

409   of sperm with malfunctioning telomeres could contribute to reduced male fertility with age.

410   Recently, sperm telomere length has been suggested as a good marker for male infertility,

411   being indicative of abnormal spermatogenesis, though its utility is still unclear [107].

412   While it is evident that telomere attrition does occur in germ cell DNA, it is still not

413   clear how these changes in gamete telomere length with parental age affect offspring.

414   Fertilisation triggers a substantial elongation of telomere lengths in the zygote, a process

415   which appears to involve the recombination-based method ALT [97, 108]. Studies to date in

416   non-human species indicate that this might not be a simple restoration to the average telomere

417   length of the parents. Most importantly in the context of this review, studies of birds, reptiles

418   and non-human mammals have reported that older fathers have offspring with shorter

419   telomeres [24, 91, 109-111], though a positive correlation between male and female age

420   within a breeding pair often makes it difficult to separate maternal and paternal effects in

421   natural populations [112]. In controlled conditions using laboratory mice, offspring from

422   older fathers have been reported to have shorter telomeres [91]. In humans on the other hand,

423   offspring from older fathers have been found to have longer telomeres [92]. In both mice and

424   men, this is in line with the respective changes in sperm telomere lengths with male age as

425   mentioned above. However, in most of the studies of parent-offspring telomere lengths in

426   humans, offspring telomere length has been measured only post-natally, often when adult,

427   and the studies are mainly cross-sectional, so multiple processes could be involved that do

428 not relate to germ cell telomere lengths. Differential survival of parental phenotypes, or

429 different environmental factors during rearing for different cohorts of fathers, could

430 potentially explain, or at least contribute to, the observed positive effect of paternal age on

431 offspring telomere length in humans. In a study in which cohort effects on changes in

432 telomere length with paternal age in humans were examined, they were found to be stronger

433 than the paternal age effect, though both were significant. Year-of-birth effects on telomere

434 length are in themselves interesting and might be due to variation in environmental

435 conditions such as nutrition and pollution which could affect the pattern of sperm ageing

436 [105]. Overall, across species, there is no consistent pattern in whether or not a paternal effect

437 on offspring telomere length is positive or negative, or absent, though positive effects appear

438 to be a feature of catarrine primates [92]. Whether this bears any relation to which sex has the

439 strongest influence on telomere length is unknown. Interestingly, there is evidence that the

440 paternal age effect in humans is detectable across at least two generations, since the age of

441 the paternal grandfather at the time of the father's conception also has a positive effect on the

442 telomere length of the grandchild [113].

443   Effects of maternal age on offspring telomere length have been relatively little studied

444 and the results are again mixed, with both positive and negative relationships being reported

445 [92, 94, 114], or alternatively no effect of the age of either parent [115, 116]. A comparison

446 in the zebra finch *Taeniopygia guttata* of offspring from the same mothers when young and

447 when old (both mated to young males) shows a marked decline in the telomere length of fully

448 grown offspring with maternal age [117]. This may or may not involve age-related changes in

449 oocytes. More longitudinal experiments involving both fathers and mothers are needed. There

450 is also a need for more studies in which early developmental stages are examined since this is

451 a very important period for phenotypic development [82, 118]. Two such studies in non-

452 humans have revealed interesting patterns. Firstly, in comparison with young males, older

453 male mice *Mus musculus* produced sperm with shorter telomeres, which led to shorter

454 telomeres in offspring at both the two-cell embryo and pup stage when that sperm was used

455 via IVF to fertilize young females [91] (Figure 2). Secondly, when the same female zebra

456 finch was mated with young and old males in quick succession (and randomized order), the

457 telomere lengths in the resulting 5-day-old embryos (held in an incubator) were shorter when

458 the father was older [29].

459   The declines in offspring telomere length with parent age potentially provide a

460 mechanism whereby offspring from older parents have reduced health and longevity. We

461   need more information on how telomere lengths are 'reset' during embryo development.

462   Inheritance of progressively shortened telomeres could act as the 'ageing factor' postulated

463   by Lansing to accumulate across generations, but experimental research is needed to examine

464   the extent to which telomere length decreases cumulatively from generation to generation

465   when only old individuals are allowed to breed. In addition, more modelling of the population

466   consequences would be very interesting given that fathers and mothers are unlikely to breed

467   only when they are very old, and will produce both more and higher quality offspring earlier

468   in their breeding lives.

469   **(c) Mitochondrial ageing in the germline**

470   Not all effects on germline ageing will be due to genomic effects. Ageing in attributes of the

471   gamete cytoplasm could also be important. Of particular interest in this context are the

472   mitochondria. Increasing mitochondrial dysfunction with age is now known to be an

473   important contributory factor to ageing of the soma [119]. Do mitochondria in germ cells also

474   age? There are a number of lines of evidence indicating that the reduced fertility in older

475   females is associated with increasing levels of dysfunction in the mitochondria of their

476   oocytes [46, 120, 121]. It has been known for a long time that mitochondria 'age' in somatic

477   tissues as a result of a gradual increase over time in levels of oxidative damage to

478   mitochondrial DNA (mtDNA) and mitochondrial membranes [122, 123] and mutations in

479   mtDNA due to replication errors [124, 125]. Oxidative damage arises as a result of an

480   imbalance between the production of ROS, principally by the mitochondria themselves, and

481   the cell's antioxidant defences [126]. This increase in damage is non-trivial: for example, the

482   level of oxidative damage and the consequent mutation rate of mitochondrial DNA is far

483   higher than that of nuclear DNA, due to mtDNA being positioned very close to the inner

484   mitochondrial membrane (the major source of the ROS) yet lacking the protective histones

485   and DNA repair capacity of nuclear DNA [122, 127]. Replication errors also accumulate

486   faster in mtDNA than in nuclear DNA due to the rapid turnover of the mitochondria [124,

487   125]. It is noteworthy that somatic cells lack the means to eliminate most forms of deleterious

488   mtDNA from their tissues [123].

489   The steady accumulation of mutations in mitochondrial DNA results in a

490   corresponding decrease over the lifetime of a cell in the efficiency with which ATP is

491   produced, and has been considered for some time to be a major contributor to the senescence

492   of somatic tissues [122, 123]. The same accumulation of damage occurs with age in the

493   mitochondria of the gametes of both sexes [46-48, 120, 128]. This is associated with impaired

494   mitochondrial function, as in somatic tissues: for instance, the ovulated oocytes of older

495   female mammals of several species have been shown to have a lower mitochondrial density

496   (measured in terms of quantity of mtDNA) and lower ATP levels, with the reduction in ATP

497   production likely to be due to lower mitochondrial membrane potentials [129-132]. A further

498   contributing factor to the decline in oocyte mitochondrial efficiency with maternal age is a

499   reduced expression of genes responsible for Coenzyme Q (CoQ), a key component of the

500   electron transport chain of the mitochondria [121]. An impressive multi-faceted study of the

501   mechanisms underlying age-related declines in fertility showed that dietary supplementation

502   with CoQ not only reversed the decline in mitochondrial function in the oocytes of ageing

503   female mice, but also increased the numbers of oocytes that these older mice ovulated and

504   restored litter sizes to those produced by young mice [121] (Figure 3). The impaired

505   mitochondrial efficiency in oocytes from older females has also been proposed as a

506   mechanism underlying the Lansing effect, if the offspring of older mothers were to inherit a

507   greater proportion of dysfunctional mitochondria which would then shorten their lives [133].

508   Contrary to expectations, some studies have found mtDNA levels (a measure of

509   mitochondrial density) to be higher (rather than lower) in the blastocysts from older women;

510   furthermore high mtDNA levels in blastocysts have been associated with a greater risk of the

511   embryo failing to implant [134, 135]. While it seems counterintuitive for high mitochondrial

512   densities to be an indicator of a failing embryo, this is in line with the 'quiet embryo'

513   hypothesis [136] that suggests normal embryonic development is associated with low rates of

514   metabolism (and hence low mitochondrial densities) at the blastocyst stage. Under this

515   scenario, the high mitochondrial copy number of oocytes from older mothers can be seen as a

516   compensatory response to their impaired mitochondrial efficiency. This has led to speculation

517   that IVF treatments could be optimised by screening for mtDNA content when embryos are

518   being selected for implantation, rejecting all those with higher mitochondrial densities [135].

519   However, follow-up studies have failed to replicate these results, finding no association

520   between the mtDNA content of early human blastocysts and either the age of the mother or

521   the likelihood of the blastocyst becoming implanted [137, 138]. At present it seems likely that

522   the discrepancies between studies are due to differences in the way in which samples are

523   either collected, stored or analysed [138, 139], and the true relationship between maternal age

524   and mitochondrial content of the early embryo remains to be resolved.

525   The relative level of oxidative damage is likely to be greater in sperm than in eggs, as
526   a consequence of sperm needing to be motile and so having a high requirement for ATP (with
527   consequent inevitable production of ROS: ROS production rates are higher in active than in
528   stored sperm [140]). In contrast, oocytes require little ATP until they start to mature; it seems
529   possible that their modest energy requirements can be met by glycolysis (not involving the
530   mitochondria) or import of ATP from neighbouring somatic cells [141, 142]. The inevitable
531   risk of more oxidative damage to sperm compared with eggs led to the 'division of labour'
532   hypothesis [141, 143], which suggested that strictly maternal inheritance of mitochondria is
533   an adaptation to avoid the transfer of damaged mitochondria to the next generation (i.e. the
534   inheritance of an aged phenotype). It has been shown in diverse species (*Drosophila*, jellyfish
535   *Auerelia aurita* and zebrafish *Danio rerio*) that the mitochondria in sperm are active,
536   producing both ATP and ROS, whereas (at least the majority of) those in primary oocytes are
537   small, quiescent and simple in structure, producing neither ATP nor ROS [128, 144, 145].
538   This division allows inheritance (via the maternal line) of undamaged 'template'
539   mitochondria, which become active in somatic and male germline tissues but not in the
540   female germline until late in oocyte development – indeed, in long-lived species such as
541   humans the mitochondria in primary oocytes can remain quiescent for up to 50 years.

542   The reduction in the number of mitochondria during gametogenesis is thought to
543   allow selection against defective mitochondria (the 'mtDNA bottleneck'), so preventing the
544   accumulation of deleterious mutations through Muller's ratchet and maintaining
545   mitochondrial function between generations [123, 146]. Once the oocyte starts to mature
546   there is intensive mitogenesis so that by the time it is fully mature it contains an order of
547   magnitude more mitochondria (and hence mtDNA) than any other cell in the body [128, 138].
548   The mitochondria become very active, so that most ATP in the oocyte is now produced
549   through oxidative phosphorylation rather than glycolysis, and there is a significant increase in
550   ATP content per oocyte [127, 146].

551   The 'division of labour' hypothesis mentioned above [141, 143] is not applicable to
552   all metazoan species. For instance, there is a group of 100+ species of bivalve molluscs,
553   spread across at least 7 families, in which the mitochondria can be inherited from either
554   parent. In this doubly-uniparental inheritance (DUI) system there are distinct 'male' M-type
555   and 'female' F-type mitochondrial lineages in the gametes that have been separately evolving
556   for some time. Both sexes usually only contain F-type mitochondria in their somatic tissues,
557   but their gametes contain only the sex-specific form of mitochondria (i.e. M-type in sperm

558 and F-type in oocytes) [147]. This means that M-type mitochondria must be passed on by
559 fathers to the germline of their sons. Moreover, both kinds of mitochondria are functionally
560 active in their respective gametes [148], so there is no division of labour and seemingly no
561 possibility for quiescent 'template' mitochondria to be passed on to the next generation [149].
562 It is thus an unresolved question as to how these DUI species manage to prevent oxidatively-
563 damaged mitochondria from being passed to offspring. The two types of mitochondria in
564 gametes have recently been shown to differ in their functioning, but in ways that would
565 actually appear to increase the risk of oxidative damage to M-type mitochondria in the sperm
566 [147]; this is so even in *Arctica islandica*, which can live to over 500 years and is thus the
567 longest lived noncolonial metazoan [150]. Whether the existence of DUI refutes the concept
568 of the 'division of labour' as a means to prevent the inheritance of an aged phenotype is
569 unresolved [149, 151].

570 Age-related changes in mitochondrial function clearly provide one potential route
571 whereby parental, particularly maternal, age can affect offspring viability, but there are still
572 unanswered questions. For instance, does the 'mtDNA bottleneck' really filter out
573 dysfunctional mitochondria? Is there any intergenerational effect of mitochondrial
574 dysfunction in ageing gametes on mitochondrial function in the offspring? Are there
575 differences in the mitochondrial activity (and hence likely build-up of ROS) in the sperm of
576 species with external versus internal fertilisation, given the relative distance that the sperm
577 have to swim? And how can a male animal live to be 500 without significant ageing of the
578 mitochondria in its sperm, or this being passed on to its sons?

579 **5. Implications for life histories**

580 It is clear that the germline itself shows age-related deterioration. Mutations can accumulate,
581 telomeres can shorten, and mitochondrial function can decline in germ cells, all of which may
582 potentially contribute to ageing of the germline and affect offspring health and longevity
583 although their relative importance is not yet known. It is also clear that in sexually
584 reproducing animals, the separation between germline and soma is not an impenetrable
585 barrier. Some information can pass epigenetically from the soma to the germ cells, and at
586 least some of this information, which generally affects gene expression, passes to the
587 resulting offspring [8, 9], with adaptive or non-adaptive consequences. The germline is not
588 entirely protected from ageing of the soma, and is also influenced by senescence of the
589 tissues that maintain the germ line. The selection pressures affecting age-related germline

590   deterioration are likely to depend on the lifespan of the species, since this will influence for

591   instance whether there has been enough time for sufficient mutations to accumulate to cause

592   dysfunction [124, 125].

593   This raises a number of interesting questions with respect to life history evolution,

594   particularly for traits such as reproductive scheduling and mating strategies. The scheduling

595   of reproduction, in terms of both the onset of sexual maturity and reproductive effort at

596   different life history stages, will be influenced by germline senescence. While older

597   individuals may have accumulated resources and experience that can have positive effects on

598   their offspring, gamete deterioration with parental age will mean that offspring produced later

599   in life could have a lower fitness value. This will give rise to a selection pressure in favour of

600   earlier reproduction and reduced reproductive effort later in life, not simply as a consequence

601   of senescence of the parental soma, but also because the offspring are inherently likely to be

602   less fit because they originate from aged germ cells. The stronger such late-life effects, the

603   greater their influence on the evolution of reproductive schedules. We should expect that

604   evolution will shape reproductive schedules towards the optimal age for reproduction but

605   there will be many other factors involved here, and many questions are unanswered. Are

606   there costs in delaying sexual maturation associated with germline deterioration? What

607   protective measures are in place for species where sexual maturation does not occur for many

608   years, and does this matter more in semelparous or iteroparous species? Does body

609   temperature affect germline deterioration, and might hibernation halt it? Why can some

610   species continue reproduction throughout a long adult life, while in others, notably humans,

611   reproduction is curtailed during adult life? Why is this more marked in females, when we

612   know that most mutations occur in the male germline and that the rate of mutation in sperm

613   DNA increases with male age?

614   Individuals do of course have the potential to reduce the effects of parental age by

615   choosing not to mate with older individuals [53, 152], provided that they can recognise the

616   age of potential mates. This could lead to conflict between the sexes, though it may not be

617   possible to disguise ageing of the soma to any significant degree. However, that individuals

618   survive into old age may be an indicator of their high genetic quality, and such individuals

619   will have acquired more experience and possibly resources. There may therefore be a conflict

620   between the resource and genetic benefits that come with an old mate and costs to offspring

621   quality from aged gametes; the balance between the two will influence mating strategies in

622   both sexes. Studies using *in vitro* fertilisation have the potential to identify effects arising

directly from gamete deterioration and to detect post-copulatory sexual selection. In houbara bustards *Chlamydotis undulata* for example, the use of sperm from older males in IVF trials gave rise to reduced hatching success and slower growing offspring [153, 154]. However, the sperm of younger males was found to either outcompete that of older males or to be preferentially selected by females [154]. Surprisingly, sperm from immature males produced the fastest growing offspring [153]; this raises the question as to why such males still behave as if they are immature when their sperm appear to be of particularly high quality, but it could relate to the costs of competition with other males.

An important consideration is the extent to which age-related germline deterioration can be prevented, slowed or reversed, or its effects mitigated. Is germline deterioration an inevitable consequence of somatic ageing, or could germline function be preserved independently of deterioration of the soma? We know little about the relationship between the respective rates of somatic and of germ cell deterioration, both among and within species. While intuitively one might expect a positive relationship, it could be negative if germ cell maintenance is costly (and there is evidence that this is so [77]). The extent to which the rate of ageing in the germline is a by-product of (and so is constrained by) the processes causing ageing in the soma is not well understood, although some of the possible mechanisms (such as dysregulation of hormonal controls) are unique to the germline.

From an evolutionary perspective, we need more research investigating the extent to which germline ageing is counteracted by the decreasing reproductive value of older individuals. The persistence of germline material over millions of years indicates that highly effective mechanisms have evolved to prevent certain cases of ageing occurring in the germline, to repair damage, and to screen gametes to eliminate damaged eggs and sperm, thereby reducing intergenerational effects at the expense of fertility. As mentioned about, offspring from older parents do show reduced fitness, and thus selection is likely to occur against breeding when the germline is aged. A life history strategy in which individuals delay reproduction to a point where the germline has deteriorated would be strongly selected against, and most reproduction occurs prior to this having occurred. An age-related decrease in the capacity of individuals to maintain a high quality germline does occur, as evidenced for example by the higher rate of genetic mutations in offspring of older parents. To some extent this could be offset by a later-life shift in investment towards gametic rather than somatic maintenance, which could increase offspring quality towards the end of parental life.

655　　However, it may either not be possible to completely prevent germline deterioration or it may
656　　simply be too costly.

657　　　　Many intriguing but tractable questions remain to be answered, and the diversity of
658　　reproductive strategies and ageing patterns amongst organisms offers a wealth of
659　　opportunities. For example, some species of Cnidaria are capable of reversing their
660　　development, turning from a sexually-mature adult into a juvenile stage without differentiated
661　　tissues, and so potentially becoming 'immortal' [155]. What are the consequences for
662　　senescence of their germline? Are the observed decreases in honey bee embryo viability with
663　　increasing age of the queen [60] due to ageing of the somatic tissues of the queen, of her
664　　oocytes or of the sperm that she has stored for much of her adult life? This could be explored
665　　by looking at changes in the performance and genome of drones produced over the course of
666　　the queen's life, since changes in these haploid castes cannot be due to sperm ageing.

667　　　　Much of the research on germline deterioration with parental age takes place in the
668　　context of mitigating age-related declines in fertility in humans and focusses on either
669　　humans or mice (notwithstanding the substantial differences in the selection pressures
670　　favouring the evolution of processes to mitigate age-related germline deterioration in long-
671　　and short-lived species). Investigation of germline senescence is a potentially very productive
672　　yet understudied field for scientists from many disciplines. A more comparative approach
673　　could greatly broaden our understanding of what is and is not possible.

674

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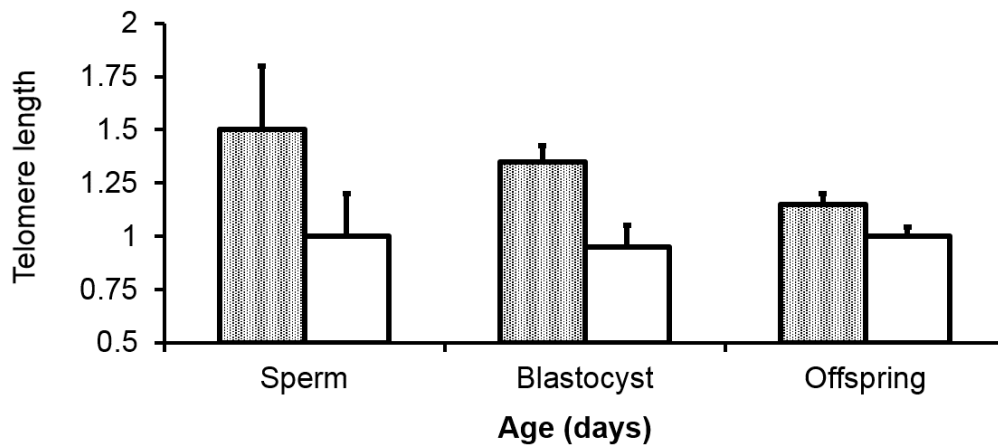1070 and plasticity. *Cell* **157**, 110-119.

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1072

1073 Figure 1:

(A)

(B)
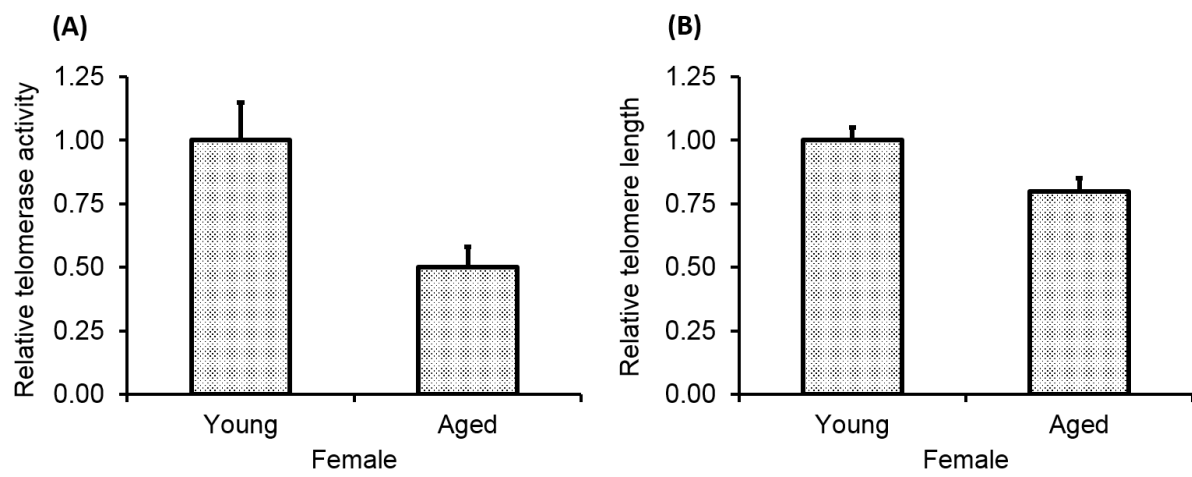
1074

1075 Fig. 1. (A) Relative telomerase activity and (B) relative telomere lengths in oocytes of young

1076 and old mice. After [98].
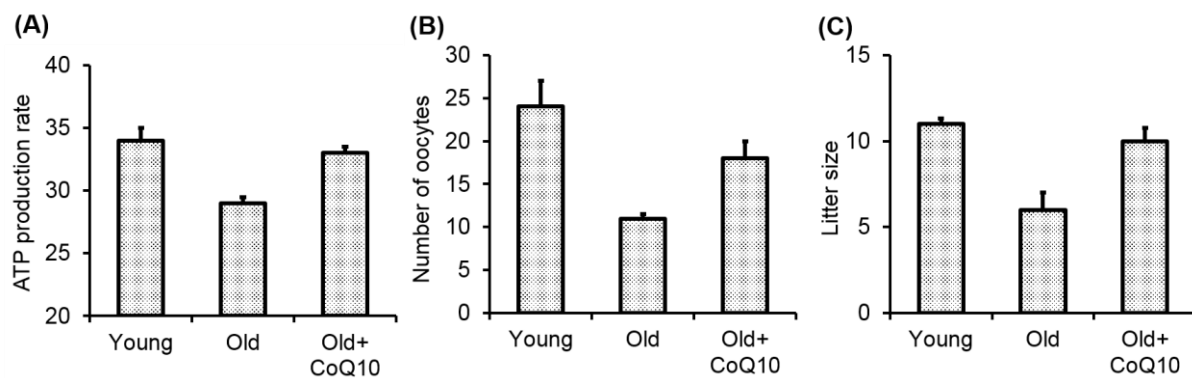
1077

1079   Figure 2:

1080

1081   Fig. 2. Differences in the relative telomere length in sperm of young (hatched bars) and old

1082   (white bars) male mice translates into differences in the telomere lengths of the resulting 2-

1083   cell blastocysts and offspring, when the sperm was used via IVF to fertilise young females.

1084   After [91].

1085

1086 Figure 3:

1087

1088 Fig. 3. (A) ATP production by oocyte mitochondria declines in old mice but is restored by

1089 supplementation with Coenzyme Q10 (CoQ10), a component of the mitochondrial electron

1090 transport chain. These changes in mitochondrial function in the oocytes are reflected in the

1091 reproductive potential of the mice: the number of oocytes (B) and offspring (C) produced

1092 after hormonal stimulation is lower in older females, but is restored after CoQ10 treatment.

1093 After [121].