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1 **Paternal age negatively affects sperm production of the progeny**

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29

30

31 **Abstract**

32 Parental age has profound consequences for offspring's phenotype. However, whether patrilineal
33 age affects offspring sperm production is unknown, despite the importance of sperm production for
34 male reproductive success in species facing post-copulatory sexual selection. Using a longitudinal
35 dataset on ejaculate attributes of the houbara bustard, we showed that offspring sired by old fathers
36 had different age-dependent trajectories of sperm production compared to offspring sired by young
37 fathers. Specifically, they produced less sperm (-48%) in their first year of life, and 14% less during
38 their lifetime. Paternal age had the strongest effect, with weak evidence for grandpaternal or great
39 grandpaternal age effects. These results show that paternal age can affect offspring reproductive
40 success by reducing sperm production, establishing an intergenerational link between aging and
41 sexual selection.

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53 **Introduction**

54 The idea that the parental phenotype can affect offspring's phenotype has been investigated in many
55 species, and there is ample evidence showing that these "parental effects" have important
56 consequences for offspring fitness and for the evolutionary dynamics of phenotypic traits (Mousseau
57 and Fox 1998; Badyaev and Uller 2009; Crean and Bonduriansky 2014).

58 Age is among the parental traits that can affect offspring phenotype (Hercus and Hoffmann
59 2000; Kern et al. 2001; Priest et al. 2002; Carnes et al. 2012; Qazi et al. 2017; Gillespie et al. 2013;
60 Lippens et al. 2017; Arslan et al. 2017). Reproductive senescence is a widespread phenomenon in
61 nature (Nussey et al. 2013; Jones et al. 2014), and involves the sequential alteration of several
62 functions from gamete production to parental care, ultimately resulting in an age-associated
63 reduction in the number of offspring produced (Lemaître and Gaillard 2017). Furthermore, negative
64 effects of aging can extend well beyond the number of offspring produced, since parental age can
65 also impinge on the performance of the progeny, including traits that are expressed at early (e.g.,
66 embryo or juvenile survival, offspring development) and late life (e.g., lifetime reproductive success
67 or longevity, the so-called Lansing effect) (Tarin et al. 2005; Garcia-Palomares et al. 2009; Lind et al.
68 2015; Bouwhuis et al. 2015; Schroeder et al. 2015; Fay et al. 2016).

69 Deleterious effects of parental age on offspring traits can be mediated by different
70 mechanisms, involving environmental, epigenetic and genetic effects (Beamonte et al. 2010; Kong et
71 al. 2012; Jenkins et al. 2014; Chen et al. 2016; Herati et al. 2017; Jónsson et al. 2017; Noguera et al.
72 2018; Bauch et al. 2019).

73 While few studies have investigated the effect of parental age on offspring lifespan and
74 lifetime reproductive success (e.g., Bouwhuis et al. 2015; Schroeder et al. 2015), even less have
75 focused on how parental age shapes the production of male gametes. In species where females mate
76 with several males during the same reproductive bout, investment into sperm production is a key
77 trait affecting male fitness (Parker and Pizzari 2010). Sperm competition and female cryptic choice
78 have been shown to be strong selective forces shaping investment into ejaculate traits and sperm

79 phenotype (Firman and Simmons 2009; Fitzpatrick and Lüpold 2014; Vuarin et al. 2019b). Therefore,
80 if parental age impinges on traits involved in post-copulatory sexual selection, this is likely to have
81 consequences for offspring reproductive success, establishing a link between aging and sexual
82 selection (Bonduriansky et al. 2008). Despite its potential ecological and evolutionary relevance, to
83 the best of our knowledge, the effect of parental age on male offspring reproductive function has
84 only been investigated in humans (Sharma et al. 2015) and inbred strains of mice (Caballero-Campo
85 et al. 2018; Xie et al. 2018). While findings generally point towards a negative effect of advanced
86 parental age on male reproductive function, no study has investigated the persistence of these
87 effects over more than two generations. However, such data might prove very valuable to indirectly
88 infer the underlying mechanisms and the possible ecological and evolutionary consequences of such
89 intergenerational effects. For instance, if parents reproducing at old age are more likely to transmit
90 *de novo* mutations to their offspring, we might expect that deleterious effects of parental age persist
91 across generations. Conversely, if parental effects arise because old parents provide different
92 environmental conditions to their offspring, or due to epigenetic changes transmitted with gametes,
93 we might expect strongest effects between parents and offspring (compared to
94 grandparents/grandoffspring or great grandparents/great grandoffspring). However, exceptions to
95 this expected pattern do exist, since epigenetic effects have been shown to persist across two
96 generations (see for instance, Eisenberg et al. 2012), and deleterious age-associated mutations of the
97 germline can be purged by negative selection.

98 Here, we took advantage of a longitudinal dataset that has been collected in a long-lived bird
99 species, the North African houbara bustard (*Chlamydotis undulata undulata*), maintained in a
100 conservation breeding program, to explore the effect of parental age on age-specific ejaculate traits
101 of the progeny. Previous work conducted on this species has shown that number of sperm and an
102 index of sperm motility improve with age and then decline as birds become older (Preston et al.
103 2011). Reproductive success of old males is also impaired, since eggs fertilized by senescing males
104 have lower hatching success, hatchlings have reduced growth, and suffer higher post-release

105 mortality (for female offspring) (Preston et al. 2015; Vuarin et al. 2019a). Moreover, post-copulatory
106 sexual selection has been shown to operate on ejaculate traits in this species (Vuarin et al. 2019a,b).

107 We first explored the effect of maternal and paternal age on offspring ejaculate traits. Since
108 we found evidence for paternal age effects on sperm production, we subsequently extended the
109 analysis including grandpaternal and great grandpaternal age, to investigate whether the effects
110 persisted through patriline.

111

112 **Materials and methods**

113 All birds used in this study are part of the Emirates Center for Wildlife Propagation (ECWP), a
114 conservation breeding program located in eastern Morocco, aiming at reinforcing natural
115 populations of the North African houbara bustard. This program relies entirely on artificial
116 inseminations (Lesobre et al. 2010).

117 Ejaculates are routinely collected using a dummy female and immediately brought into an
118 adjacent laboratory where the proportion of motile sperm, the number of sperm, the volume and
119 the proportion of morphologically normal sperm in the ejaculate are assessed (see online material
120 for details). Females are regularly checked for reproductive status and inseminated with freshly
121 collected semen when ready to lay. Laid eggs are collected every morning and incubated following a
122 standard protocol.

123 We investigated the potential effect of parental age on the following ejaculate traits: i) the
124 mean number of sperm in the ejaculate per breeding season, ii) the mean mass motility index per
125 breeding season, iii) the mean proportion of sperm with normal morphology in the ejaculate per
126 breeding season. Data on age-specific sperm quantity and mass motility index were available for
127 1,708 individuals, from 1 to 17 years old, born from 2002 to 2018 and collected between 2003 and
128 2019. These 1,708 individuals were produced by 989 dams and 682 sires. The overall data set
129 included 11,107 observations. Dam age ranged from 1 to 13 years (mean \pm sd = 3.78 \pm 1.85), and sire
130 age from 1 to 14 years (mean \pm sd = 3.88 \pm 1.90). Owing to small sample size, individuals older than

131 15 years were grouped in the same age class; similarly, dams and sires aged from 10 to 14 years were
132 grouped in the same age class. The correlation coefficient between maternal and paternal age was
133 low ($r = 0.095$, $n = 1,708$), showing that the two variables convey independent information on
134 parental age. Data on the proportion of normal sperm were available for a smaller number of
135 individuals (1,270 individuals produced by 758 dams and 543 sires, for a total of 2,273 observations).

136 The overall data set only included birds for which paternal identity (and therefore paternal
137 age at fertilization) was certain. This was ensured because females were either inseminated with the
138 semen of a single male, or in case of multiple inseminations with different males, microsatellite
139 markers were used to perform parentage analyses and identify the sire (Lesobre et al. 2010).

140

141 *Statistical analyses*

142 We used general additive models (GAM) to infer the shape of age-dependent variation of
143 each of the three ejaculate attributes considered here. GAM is a nonparametric regression that
144 relaxes the assumption of linearity and allows to explore traits that vary non-linearly without the
145 constraints that are usually associated with polynomial models.

146 We used linear mixed models (LMM) to explore the effect of maternal and paternal age on
147 age-dependent variation of ejaculate traits of the offspring. We fitted separate models for each
148 response variable [i.e., number of sperm in the ejaculate (log-transformed), mass motility index, and
149 the proportion of sperm with normal morphology]. All models had the same structure (normal
150 distribution of errors), with the following terms fitted as fixed effects: offspring age (log-
151 transformed), squared offspring age, maternal age, and paternal age. We also fitted the two-way
152 interactions of parental age with offspring age and squared offspring age to test whether age-
153 dependent trajectories were affected by parental age. The random effects included offspring,
154 maternal and paternal identities, year of birth and year of data collection. If individuals with
155 particular phenotypic traits enter or exit from the sampled population at different ages (selective
156 appearance and disappearance), this might generate a pattern of age-dependent variation at the

157 population level, potentially leading to erroneous conclusions about the age-dependent trajectories
158 at the individual level. To infer the within-individual variation with age, independently from the
159 population-level (between-individual) variation caused by selective appearance and/or
160 disappearance, we followed the methods advocated by van de Pol and Verhulst (2006), and included
161 age at first insemination (that determines at which age a given individual enters the breeding
162 population), and age at last insemination (that determines at which age a given individual exits the
163 breeding population) as fixed effects. We started with the model that included all single effects and
164 interactions and we sequentially dropped those that were not statistically significant, starting first
165 with interactions and then with single effects, until obtaining a minimal adequate model. Note that
166 age at first and last insemination were always kept in the models.

167 To investigate the effect of patrilineal age (over three generations) on sperm production, we
168 used two approaches. First, we grouped birds according to the age of their fathers, grandfathers and
169 great grandfathers. Ascendants that bred between 1 and 5 years were considered young, ascendants
170 that bred when older than 5 years were considered old. Therefore, birds that consistently had young
171 (y) ascendants were considered to have a “yyy” patrilineal age (the first letter referring to paternal
172 age, the last letter to great grandpaternal age); birds that consistently had old (o) ascendants were
173 considered to have a “ooo” patrilineal age. Two age classes and three generations give 8 possible
174 patrilineal age groups (yyy, yyo, yoo, ooo, oyy, ooy, yoy, oyo). The effect of patrilineal age on sperm
175 production was assessed running 6 LMMs where different patrilineal age groups were contrasted.
176 Due to small sample size, we could not include ooy, oyo, ooy, ooo groups in the models. Each model
177 had the same structure, with offspring age and squared age, patrilineal age (plus interactions), age at
178 first and age at last insemination included as fixed effects. The random effects included offspring
179 identity, maternal and paternal identity, year of birth and year of data collection. Restricting the
180 sample size to individuals with known father, grandfather and great grandfather reduced the number
181 of individuals included in the model to 888, for a total of 5,504 observations. Second, we ran a LMM
182 where the three ascendant ages were included in the same model, as continuous variables. This

183 model therefore relaxed the constraint associated with the cutoff used to categorize birds as young
184 or old. The model included offspring identity, maternal and paternal identity, year of birth and year
185 of data collection as random effects.

186 GAMs and LMMs were performed using PROC GAM and PROC MIXED (SAS, 14.3),
187 respectively.

188

189 **Results**

190 The GAMs showed that both the number of sperm in the ejaculate (i.e., sperm production),
191 and the mass motility index increased up to the age of 5 years and then gradually declined as
192 individuals became older, with a strong quadratic pattern (number of sperm in the ejaculate: $\chi^2 =$
193 582.04, df = 3, n = 11,107; p < 0.0001; mass motility index: $\chi^2 = 413.47$, df = 3, n = 11,107, p < 0.0001;
194 fig. S1A,B). The proportion of morphologically normal sperm showed a more complex pattern of
195 variation with age, suggesting a higher-order behavior and a later peak value (7 years, $\chi^2 = 22.87$, df =
196 3, n = 2,273, p < 0.0001; fig. S1C).

197 The LMM performed on the number of sperm in the ejaculate showed that offspring age-
198 dependent trajectory was modulated by paternal age, as indicated by the highly significant
199 interactions between paternal and offspring age (both linear and quadratic components, table 1).
200 Maternal age was not a significant predictor of sperm production, nor of its age-dependent variation
201 (table 1).

202 Grouping paternal age into two age classes [up to the peak age (i.e., from 1 to 5 years) and
203 post-peak age (i.e., older than 5 years)], allowed to visualize the effect of sire age on the trajectories
204 of age-dependent offspring sperm production (fig. 1; table S1). Offspring sired by old fathers had a
205 lower sperm production (-16.9% difference in LS-MEANS on back-transformed values) during their
206 early life (1-5 years) compared to offspring sired by young fathers (LMM, paternal age: t = -2.78, p =
207 0.0055, age at first insemination: t = 0.97, p = 0.3299; age at last insemination: t = 19.44, p < 0.0001;
208 number of observations = 5,640). The difference was very strong at the age of 1, with offspring sired

209 by old fathers showing a 47.9% reduction in their sperm production (LMM, paternal age: $t = -2.52$, $p = 0.0122$, age at last insemination: $t = 1.48$, $p = 0.1388$; number of observations = 401; fig. 2A). During
210 = 0.0122, age at last insemination: $t = 1.48$, $p = 0.1388$; number of observations = 401; fig. 2A). During
211 the post-peak age (over 5 years), offspring sired by old fathers still had a lower sperm production (-
212 7.6%) compared to offspring sired by young fathers, but this difference was not statistically
213 significant (LMM, paternal age: $t = -1.11$, $p = 0.2651$, age at first insemination: $t = 0.54$, $p = 0.5907$;
214 age at last insemination: $t = 8.41$, $p < 0.0001$; number of observations = 5,467). Nevertheless, over
215 the entire lifespan, offspring sired by old fathers produced 14% less sperm per ejaculate than
216 offspring sired by young fathers (LMM, paternal age: $t = -2.19$, $p = 0.0283$, age at first insemination: t
217 = 2.64, $p = 0.0083$; age at last insemination: $t = 16.55$, $p < 0.0001$; number of observations = 11,107;
218 fig. 2B).

219 Given that sperm number and mass motility index positively covaried ($r = 0.736$, $n = 11,107$),
220 we ran an additional LMM where the latter variable was included as a covariate. This model
221 therefore aimed to investigate whether the effect of paternal age on sperm number still holds when
222 keeping mass motility index constant. The model provided similar results than the previous one
223 (table S2), suggesting that the effect of parental age on sperm number did not arise because of the
224 covariation between sperm number and motility.

225 We also found that paternal age affected the age-dependent variation in offspring mass
226 motility index [significant paternal age x offspring age (and squared age); table S3]. However, this
227 pattern was entirely driven by the covariation with the number of sperm in the ejaculate (table S4).
228 As for number of sperm in the ejaculate, maternal age had no effect on mass motility index, nor on
229 its age-dependent variation (table S3).

230 Neither paternal nor maternal age modulated the proportion of morphologically normal
231 sperm in the ejaculate or its age-dependent trajectory (table S5).

232 To go further, we investigated if the paternal age effect on sperm production persisted over
233 generations. We grouped birds according to the age of their father, grandfather and great
234 grandfather and ran separate models contrasting different patrilineal ages (table 2). Overall, we

235 found that paternal age had the strongest effect on sperm production. For instance, comparing
236 sperm production of birds with young or old fathers, but young grandfathers and great grandfathers,
237 showed a marked reduction in early production of sperm in birds sired by old fathers (table 2, fig. S2).
238 Unfortunately, some of the patrilineal age groups had very few birds making impossible to run all
239 possible comparisons. Nevertheless, comparing birds that only differed in the great grandpaternal
240 age class (yyy vs. yyo), also showed a negative effect of great grandpaternal age on sperm production
241 (table 2, fig. S3), suggesting a persisting effect of paternal age over generations. A seemingly
242 contradictory result came from the comparison between birds with young ascendants over the three
243 generations (yyy) and birds with old grandfathers and great grandfathers (yoo). Indeed, in this
244 comparison, yoo birds had the highest sperm production (table 2). It should be noted however, that
245 the number of birds in this patrilineal age group was particularly small, compared to the yyy group
246 (34 vs. 519), and as such the result of this model should be taken with caution.

247 Running a LMM where paternal, grandpaternal and great grandpaternal age were included in
248 the same model as continuous variables provided similar results to those reported above, with
249 paternal age having the strongest effect on offspring sperm production (table S6).

250

251 **Discussion**

252 We found that paternal but not maternal age affected sperm production of the progeny, and
253 that this effect was the strongest over the one-generation transmission. Other ejaculate traits (mass
254 motility index and the proportion of sperm with a normal morphology) were not affected by parental
255 age.

256 Although the effect of parental age on offspring phenotype has been studied in several
257 systems, most of this previous work has focused on maternal age, because it was believed that
258 mothers had higher control over offspring phenotype. This view is, however, rapidly changing, and
259 evidence is accumulating i) showing that paternal age is associated with several offspring phenotypic
260 traits (Garcia-Palomares et al. 2009; Fay et al. 2016; Arslan et al. 2017; Caballero-Campo et al. 2018;

261 Wylde et al. 2019); ii) identifying the possible underlying mechanisms (Curley et al. 2011; Kong et al.
262 2012; Soubry et al. 2014; Xie et al. 2018).

263 Assessing male reproductive function is a hard task in free-ranging animals because this
264 requires repeated samples of ejaculates. Recent work has shown that paternal age negatively
265 impinges on lifetime reproductive success of male house sparrows (*Passer domesticus*) (Schroeder et
266 al. 2015), suggesting that one or several components of the male reproductive function are impaired
267 when individuals are sired by an old father. Our results suggest that reduced gamete production
268 might be one of the physiological mechanisms underlying the negative effect of paternal age on male
269 offspring lifetime reproductive success, and especially so in species where males face post-
270 copulatory sexual selection through sperm competition. This might be particularly relevant when
271 paternal age affects the production of gametes during the early life of the descendants, when the
272 force of selection is at its highest value. Number of gametes transferred during the mating is an
273 important driver of siring success and especially so in species where females mate with several males
274 and ejaculates compete for egg fertilization (Parker and Pizzari 2010). In the houbara bustard, we
275 have previously shown that ejaculate attributes, such as motility, improve male siring success during
276 competitive fertilizations (when sperm from different males compete within the female reproductive
277 tract) (Vuarin et al. 2019b). Similarly, when females are inseminated with ejaculates of two males,
278 the probability of siring success of the last male (last male precedence) decreases as the number of
279 sperm of the first male used to inseminate the female increases (L. Lesobre, unpublished results).
280 Therefore, the finding that paternal age impinges on sperm production has potentially far reaching
281 ecological and evolutionary consequences. At the ecological level, parental age effects can be a
282 phenotypic source of variation contributing to shape offspring quality and life history traits, with
283 possible consequences in terms of population dynamics. In agreement with this hypothesis,
284 experimental work conducted on soil mites (*Sancassania berlesei*) has shown that changes in life
285 history traits (longer developmental time and larger size at maturity) due to maternal age lead to
286 different population dynamics that persist over generations (Benton et al. 2008). At the evolutionary

287 level, negative parental age effects on the progeny contribute to the age-associated decline of fitness
288 and increase the strength of selection for earlier reproduction (Priest et al. 2002). Paternal age
289 effects on the progeny might also alter the shape of the trade-off between early and late trait
290 expression. One of the best supported predictions of the theory on the evolution of senescence is
291 that high investment into early expressed traits comes at the price of reduced investment in late life,
292 resulting in a negative correlation between early and late traits (Lemaître et al. 2015). However,
293 offspring produced by old parents might have compromised quality and have impaired expression of
294 fitness related traits over their entire reproductive life, flattening the relationship between early and
295 late life traits. Finally, the extent of the costs associated with mating with an old partner also
296 suggests that selection might have promoted mechanisms of avoidance of senescing males. In
297 agreement with this hypothesis, we recently showed that female houbara bustards concomitantly
298 inseminated with sperm from males at prime and post-prime age had a higher proportion of their
299 eggs fertilized by prime age males (Vuarin et al., 2019a). This finding, therefore, suggests that post-
300 copulatory selection might allow them to avoid the cost of being fertilized by senescing males.

301 Whether such paternal age effects persist over generations remains an open question. We
302 only found a moderate evidence for a negative effect of great grandparental age on sperm
303 production, and the comparison between birds produced by consistently young ascendants and birds
304 produced by young fathers and old grandfathers and great grandfathers, showed the opposite
305 pattern (higher sperm production for the latter group). These results should be interpreted with
306 caution, due to the heterogeneity in sample size (number of birds) in each patrilineal age group.

307 In species where males only contribute to the reproduction through their ejaculate, paternal
308 age effects might arise because of age-associated changes in ejaculate attributes (Sharma et al.
309 2015). These effects have been named ejaculate-mediated paternal effects and can involve purely
310 environmental, epigenetic or genetic mechanisms (see Immler 2018 and Evans et al. 2019 for recent
311 reviews). During aging, both the cellular and non-cellular fraction of the ejaculate undergo profound
312 changes that have the potential to further affect offspring phenotype. Sperm are particularly

313 sensitive to oxidant attack which might compromise DNA integrity (Aitken 2020). Antioxidants
314 contained in seminal plasma play a crucial role for the preservation of sperm DNA integrity (Wai-sum
315 et al. 2006) and during aging some of this protective function might be lost. Epigenetic changes can
316 also occur in male germline cells (Xavier et al. 2019). DNA methylation in sperm is associated with
317 advanced male age (Jenkins et al. 2014), can be transmitted to the progeny and affect offspring
318 phenotype (Xie et al. 2018). However, epigenetic reprogramming can reverse methylation patterns
319 (Law and Jacobsen 2010; Rando and Chang 2012; Zhang et al. 2020), resetting the system and
320 explaining why epigenetic effects can be lost across generations. Other epigenetic factors such as
321 histone modification, noncoding RNAs contained in the seminal plasma, or inheritance of shortened
322 telomeres can also be at play (Chen et al. 2016; Immler 2018; Bauch et al. 2019). Finally, male
323 germline is particularly prone to accumulate *de novo* mutations due to the process of cell division
324 that occurs throughout the entire reproductive life of males. In agreement with this hypothesis,
325 paternal age and the length of male reproductive life are good predictors of the number of *de novo*
326 mutations passed on to the progeny in primates (Thomas et al. 2018); and inheritance of age-
327 associated *de novo* mutations has been identified as a possible risk factor for several offspring
328 pathologies (e.g., Kong et al. 2012). In addition to the sperm nuclear contribution to the offspring,
329 abnormalities in sperm centrioles can also affect offspring phenotype, although evidence has mostly
330 been reported in humans (Avidor-Reiss et al. 2019).

331 Our experimental design allows us to discard several other possible causes of the negative
332 parental age effects on sperm production. A common confounding factor in studies of parental age
333 effects derives from the possible differential investment of females according to the perceived
334 phenotypic quality of mates (Sheldon 2000). If old partners are perceived as poor mates, females
335 might adaptively adjust their investment into eggs or offspring care, producing an apparent
336 association between father age and offspring phenotype. However, reproduction of houbara
337 bustards kept in the captive breeding program is entirely based on artificial inseminations which
338 implies that i) the paternal contribution is restricted to the ejaculate; ii) females are never in contact

339 with males and therefore cannot adjust their investment into eggs according to male phenotype; iii)
340 eggs are collected as soon as they are laid and thus there is no maternal care that might
341 subsequently affect offspring quality. This last point might explain why we did not find any evidence
342 for maternal age effects, if such effects occur through differential environmental conditions
343 experienced by offspring of old vs. young mothers (Beamonte-Barrientos et al. 2010; Plaistow et al.
344 2015).

345 To conclude, we showed that paternal but not maternal age reduced sperm production in
346 offspring. These delayed paternal effects have the potential to affect the strength of selection
347 operating on post-copulatory traits and male reproductive fitness in the wild, establishing a link
348 between ageing and sexual selection.

349

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358

359 **Competing interest statement**

360 The authors declare no competing interest.

361

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495 Zhang, W., Qu, J., Liu, G.-H. & Belmonte, J.C.I. (2020). The ageing epigenome and its rejuvenation.
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497 Table 1. Linear mixed effects model exploring the effect of parental age on the number of sperm in
 498 the ejaculate (log-transformed). The model included offspring age (log-transformed), squared
 499 offspring age, maternal age, paternal age, the interactions between offspring age and parental age,
 500 and age at first and last insemination as fixed effects. Offspring, maternal and paternal identities,
 501 year of birth and year of data collection were included as random effects. The analysis was based on
 502 11,107 observations collected over 17 years on 1,708 individuals (produced by 989 dams and 682
 503 sires over 17 cohorts). We report parameter estimates (with SE and 95% CI), t and p values for the
 504 initial model and the minimal adequate model.
 505

Initial model					
<i>Fixed effects</i>	<i>Parameter estimate</i>	<i>SE</i>	<i>t</i>	<i>p</i>	<i>95% CI</i>
Intercept	0.491	0.306			
Age at first insemination	-0.009	0.025	-0.37	0.7109	-0.057/0.039
Age at last insemination	0.180	0.010	17.95	<0.0001	0.161/0.200
Offspring age	1.538	0.146	10.52	<0.0001	1.251/1.824
Offspring age ²	-0.774	0.056	-13.85	<0.0001	-0.883/-0.664
Dam age	-0.001	0.022	-0.03	0.9739	-0.044/0.043
Sire age	-0.107	0.022	-4.84	<0.0001	-0.150/-0.064
Offspring age x Dam age	-0.010	0.027	-0.36	0.7209	-0.063/0.043
Offspring age x Sire age	0.130	0.028	4.73	<0.0001	0.076/0.184
Offspring age ² x Dam age	0.005	0.009	0.48	0.6306	-0.014/0.023
Offspring age ² x Sire age	-0.038	0.010	-3.97	<0.0001	-0.057/-0.019
<i>Random effects</i>	<i>Variance</i>	<i>SE</i>	<i>z</i>	<i>p</i>	
Individual ID	0.574	0.038	14.91	<0.0001	
Dam ID	0.107	0.031	3.41	0.0003	
Sire ID	0.030	0.020	1.47	0.0713	
Year of birth	0.014	0.010	1.42	0.0778	
Year of data collection	1.177	0.444	2.65	0.0040	
Residual	0.521	0.008	67.76	<0.0001	

506

Minimal adequate model					
<i>Fixed effects</i>	<i>Parameter estimate</i>	<i>SE</i>	<i>t</i>	<i>p</i>	<i>95% CI</i>

Intercept	0.489	0.295			
Age at first insemination	-0.009	0.025	-0.38	0.7006	-0.058/0.039
Age at last insemination	0.180	0.010	17.95	<0.0001	0.160/0.200
Offspring age	1.509	0.113	13.36	<0.0001	1.287/1.730
Offspring age ²	-0.758	0.046	-16.54	<0.0001	-0.848/-0.669
Sire age	-0.106	0.022	-4.83	<0.0001	-0.150/-0.063
Offspring age x Sire age	0.129	0.027	4.70	<0.0001	0.075/0.183
Offspring age ² x Sire age	-0.038	0.010	-3.93	<0.0001	-0.057/-0.019
<i>Random effects</i>	<i>Variance</i>	<i>SE</i>	<i>z</i>	<i>p</i>	
Individual ID	0.573	0.038	14.93	<0.0001	
Dam ID	0.107	0.031	3.42	0.0003	
Sire ID	0.030	0.020	1.46	0.0721	
Year of birth	0.014	0.010	1.42	0.0775	
Year of data collection	1.165	0.439	2.66	0.0039	
Residual	0.521	0.008	67.77	<0.0001	

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508 Table 2. Linear mixed effects models exploring the effect of patrilineal age (paternal, grandpaternal
509 and great grandpaternal ages) on the number of sperm in the ejaculate (log-transformed). We ran 6
510 models contrasting patrilineal ages (yyy vs. oyy; yyy vs. yyo; yyy vs. yoo; yoo vs. oyy; yyo vs. yoo; yoo;
511 oyy; the first letter referring to the paternal age, the second letter to the grandpaternal age, the third
512 letter to great grandpaternal age). Offspring age (log-transformed), squared offspring age, patrilineal
513 age, the interactions between offspring age and patrilineal age, age at first and last insemination
514 were included as fixed effects. Offspring, maternal and paternal identities, year of birth and year of
515 data collection were included as random effects. The analysis was based on 5,504 observations
516 collected over 15 years on 888 males (produced by 624 dams and 394 sires over 16 cohorts). We
517 report parameter estimates (with SE and 95% CI), t and p values. Only the results of the fixed effects
518 are reported.

<i>Fixed effects</i>	<i>Parameter estimate</i>	<i>SE</i>	<i>t</i>	<i>p</i>	<i>95% CI</i>
<i>yyy vs. oyy</i>					
Intercept	0.161	0.328			
Age at first insemination	-0.010	0.046	-0.21	0.8324	-0.100/0.081
Age at last insemination	0.244	0.021	11.68	<0.0001	0.203/0.285
Offspring age	1.729	0.099	17.42	<0.0001	1.534/1.923
Offspring age ²	-0.881	0.057	-15.36	<0.0001	-0.993/-0.768
Patrilineal age (oyy)	-0.949	0.210	-4.52	<0.0001	-1.360/-0.538
Offspring age x patrilineal age (oyy)	1.127	0.276	4.08	<0.0001	0.586/1.669
Offspring age ² x patrilineal age (oyy)	-0.287	0.103	-2.79	0.0053	-0.489/-0.085
<i>yyy vs. yyo</i>					
Intercept	0.442	0.288			
Age at first insemination	-0.019	0.040	-0.48	0.6288	-0.097/0.058
Age at last insemination	0.205	0.018	11.28	<0.0001	0.169/0.241
Offspring age	1.679	0.095	17.75	<0.0001	1.494/1.865
Offspring age ²	-0.817	0.050	-16.38	<0.0001	-0.915/-0.719
Patrilineal age (yyo)	-0.434	0.140	-3.11	0.0019	-0.708/-0.161
Offspring age x patrilineal age (yyo)	0.484	0.160	3.02	0.0025	0.170/0.798
Offspring age ² x patrilineal age (yyo)	-0.120	0.055	-2.18	0.0292	-0.228/-0.012
<i>yyy vs. yoo</i>					
Intercept	0.305	0.311			
Age at first insemination	-0.01	0.047	-0.20	0.8390	-0.102/0.083
Age at last insemination	0.224	0.021	10.84	<0.0001	0.184/0.265
Offspring age	1.671	0.096	17.39	<0.0001	1.483/1.859
Offspring age ²	-0.827	0.055	-15.00	<0.0001	-0.935/-0.719
Patrilineal age (yoo)	0.890	0.293	3.04	0.0024	0.315/1.465
Offspring age x patrilineal age (yoo)	-0.901	0.356	-2.53	0.0113	-1.599/-0.204
Offspring age ² x patrilineal age (yoo)	0.278	0.132	2.10	0.0356	0.019/0.538

<i>yyo vs. oyy</i>					
Intercept	0.719	0.331			
Age at first insemination	-0.049	0.060	-0.82	0.4135	-0.166/0.068
Age at last insemination	0.119	0.030	3.93	<0.0001	0.059/0.178
Offspring age	2.214	0.158	14.03	<0.0001	1.904/2.523
Offspring age ²	-0.667	0.063	-10.57	<0.0001	-0.791/-0.544
Patrilineal age (oyy)	-0.679	0.240	-2.83	0.0047	-1.150/-0.209
Offspring age x patrilineal age (oyy)	0.736	0.302	2.44	0.0147	0.145/1.328
Offspring age ² x patrilineal age (oyy)	-0.211	0.110	-1.91	0.0566	-0.427/0.006
<i>yyo vs. yoo</i>					
Intercept	1.329	0.241			
Age at first insemination	-0.090	0.057	-1.59	0.1130	-0.201/0.021
Age at last insemination	0.028	0.022	1.29	0.1967	-0.015/0.071
Offspring age	2.139	0.148	14.43	<0.0001	1.848/2.430
Offspring age ²	-0.640	0.059	-10.94	<0.0001	-0.755/-0.526
Patrilineal age (yoo)	1.317	0.298	4.42	<0.0001	0.732/1.902
Offspring age x patrilineal age (yoo)	-1.286	0.367	-3.51	0.0005	-2.006/-0.567
Offspring age ² x patrilineal age (yoo)	0.337	0.135	2.49	0.0129	0.071/0.602
<i>yoo vs. oyy</i>					
Intercept	1.667	0.514			
Age at first insemination	0.005	0.104	0.05	0.9596	-0.200/0.210
Age at last insemination	0.134	0.051	2.63	0.0087	0.034/0.235
Offspring age	1.473	0.405	3.64	0.0003	0.678/2.269
Offspring age ²	-0.581	0.165	-3.52	0.0005	-0.905/-0.257
Patrilineal age (oyy)	-1.859	0.361	-5.16	<0.0001	-2.567/-1.151
Offspring age x patrilineal age (oyy)	1.760	0.454	3.88	0.0001	0.868/2.652
Offspring age ² x Patrilineal age (oyy)	-0.443	0.170	-2.61	0.0093	-0.776/-0.110

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527 **Figure legends**

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529 Figure 1. Age-dependent trajectories of offspring sperm number in the ejaculate (log-transformed
530 $\times 10^6$) as a function of paternal age. A) We report the raw data (blue boxes = 1-5 years old; red boxes
531 = over 5 years old) and B) the LMM predictions. Data on the x-axis were slightly jittered to improve
532 readability.

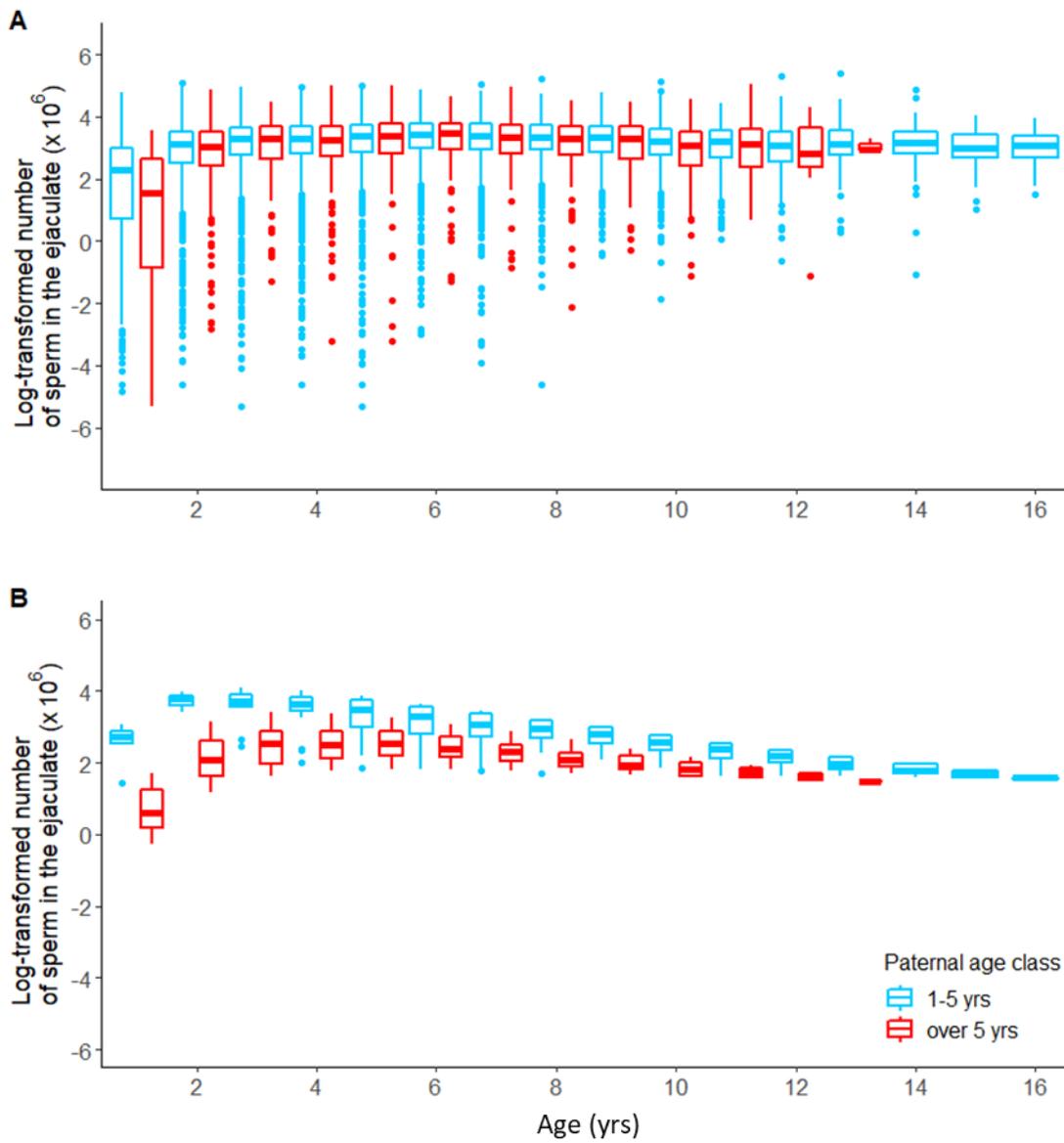
533

534 Figure 2. A) Least-square means (\pm SE) of number of sperm in the ejaculate (log-transformed $\times 10^6$) at
535 the age of 1 year, for the two paternal age classes. B) Least-square means (\pm SE) of number of sperm
536 in the ejaculate (log-transformed $\times 10^6$) over the whole life, for the two paternal age classes.

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539 Figure 1.



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547 Figure 2.

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