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► To cite this version:

Mathieu Douhard, Victor Ronget, Frédéric Douhard. DOHaD: A Menagerie of Adaptations and Perspectives: Long-lasting effects of the presence of male siblings in utero on subsequent reproductive performance. *Reproduction* [Cambridge], 2024, 167 (3), pp.e230049. 10.1530/rep-23-0049. hal-04685562

HAL Id: hal-04685562

<https://hal.science/hal-04685562v1>

Submitted on 10 Sep 2024

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1 Long-lasting effects of the presence of male siblings in utero on
2 subsequent reproductive performance

3

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11 Type of article: Review

12 Short title: Litter sex composition and reproduction

13 Keywords: co-twin sex, sex hormones, litter sex ratio, reproduction, viviparity

14 Word count: 5018 (excluding references, box, figure and table legends)

15 **Abstract**

16 Laboratory studies with rodents indicate that in utero proximity of a female to male foetus
17 can affect female's subsequent reproduction due to elevated testosterone exposure during
18 early development. It remains unknown whether these findings can be generalised to non-
19 laboratory species because the need for caesarean section makes it difficult to determine the
20 intrauterine position outside laboratory conditions. As an alternative, some studies have
21 compared the reproductive performance of individuals born in male-biased litters to those
22 born in female-biased litters. We identified 44 of those studies in 28 viviparous species for a
23 total of 176 relationships between litter sex composition around the time of birth and
24 subsequent reproductive performance (fertility, fecundity, age at first reproduction, interbirth
25 intervals or post-natal survival of offspring). Those relationships are discordant and complex
26 both within and across species. Some factors can mask an actual association between litter
27 sex composition and reproductive performance. Conversely, a part of significant relationships
28 between litter sex composition and reproductive performance likely arises via pathways other
29 than androgen- and oestrogen-transfer between fetuses of different sexes.

30 **In brief**

31 In litter-bearing species, developing offspring can be exposed to different concentrations of
32 androgens and oestrogens according to the sex of neighbouring fetuses. However, the
33 relationships between litter sex composition and subsequent reproductive performance are
34 discordant and complex.

35 **Introduction**

36 The 'developmental origins of health and disease' (DOHaD) hypothesis posits that events
37 experienced during critical periods of development, and particularly during intrauterine life,
38 have profound and long-term consequences on health (Gluckman et al., 2010). Initially
39 focused on the association between foetal exposure to maternal undernutrition and increased
40 risk of cardiovascular diseases in adults (Barker et al., 1989), DOHaD studies now include a
41 wide range of non-communicable diseases such as fertility problems (Jazwiec & Sloboda,
42 2019). Environmental factors explain most of the observed variation in subfertility for women
43 as well as men (Ahrenfeldt et al., 2020). In particular, exposure to endocrine-disrupting
44 chemicals (EDCs) during foetal development is associated with altered fertility and fecundity
45 (Green et al., 2021). Most of these substances are able to cross the placenta, interfere with
46 androgen and oestrogen synthesis, secretion or metabolism to alter the action of these sex
47 hormones and lead to long-latency adverse outcomes (Green et al., 2021; Vaiserman, 2014).

48 Natural hormonal perturbations during early development can also have long-term
49 consequences on fertility and fecundity, similar to the effects of EDCs. The most extreme
50 illustration of such effects is the freemartin syndrome in domesticated bovids (Iannuzzi et al.,
51 2021; Padula, 2005). A freemartin is a sterile female that shows masculinisation of the
52 reproductive tract to varying degrees due to the presence of a male co-twin in utero. This
53 syndrome arises when the placentae of male and female twins establish vascular connections
54 enabling the passage of cells and hormones involved in sexual differentiation (e.g. anti-
55 Mullerian hormone and testosterone) from a male foetus to the female foetus. Genetically, a
56 freemartin is chimeric with lymphocytes showing XX/XY chromosomes. Freemartinism
57 concerns a vast majority (between 82 and 92%) of female cows born co-twin to a male (Esteves

58 et al., 2012; Zhang et al., 1994). In addition, some freemartins are from single births because
59 of death of their male co-twin in utero (Iannuzzi et al., 2021; Padula, 2005). Freemartinism is
60 mostly reported in cattle, although it is increasing in prevalence in sheep populations under
61 selection for increased litter size (Padula, 2005).

62 Less extreme, but far more common, is the influence of intrauterine position (IUP) on
63 subsequent reproduction in laboratory rodents. During sexual differentiation in mammals,
64 male foetuses have higher amounts of serum testosterone than females, whereas in some
65 species, such as house mice, females have greater serum concentrations of oestradiol than
66 males (vom Saal, 1989a). These hormones can transfer among adjacent foetuses through the
67 amniotic fluid (Even et al., 1992). As a result, foetuses positioned between two males in the
68 uterus (2M females and 2M males) have greater concentrations of serum testosterone
69 whereas those positioned between two females (2F females and 2F males) can have greater
70 concentrations of serum oestradiol relative to other foetuses of the same sex (Ryan &
71 Vandenberg, 2002; vom Saal, 2016). Differences in sex hormone concentration due to IUP
72 are relatively small (Vandenberg et al., 2012). For example, in house mice, the difference in
73 serum concentration of testosterone and oestradiol between 2M and 2F females is 1 ng/mL
74 and 40 pg/ml, respectively (vom Saal, 2016). However, very small differences in sex hormones
75 due to IUP are associated with marked effects on morphological, behavioural and
76 reproductive traits (Ryan & Vandenberg, 2002). For example, 2M female mice have
77 masculinised traits, including relatively long anogenital distances, delayed puberty, increased
78 aggression, decreased attractiveness, earlier age at cessation of fertility compared to 2F
79 females (Rines & vom Saal, 1984; vom Saal, 1989b; vom Saal & Bronson, 1978; vom Saal &
80 Moyer, 1985). In addition, the IUP of a female can influence the sex ratio of her own litters,
81 with 2M female mice and gerbils producing more males and 2F females producing more

82 females (Clark & Galef, 1995; Vandenberg & Huggett, 1994). The consequences of IUP are
83 not limited to females (vom Saal, 2016; vom Saal et al., 1983). For example, 2M gerbil males
84 are more attractive to females and sire more offspring than do 2F males (Clark et al., 1992).
85 Interestingly, effects of IUP on adult phenotype and reproduction of males and females vary
86 from one species to the next. Vom Saal et al. (1999) hypothesised that such variability in IUP
87 effects reflect differences in the way that testosterone and oestradiol regulate development
88 in different species.

89 It has been proposed that the IUP phenomenon could be an evolutionary response to
90 environmental variability (vom Saal, 1981, 1989a). By diversifying the phenotypes of a litter
91 via differential sex hormone exposures, a mother could ensure that a least one of her offspring
92 matches the future environmental conditions. For example, 2F females may have better
93 reproductive success when resources are plentiful, but 2M females may be more successful
94 breeders under stressful conditions because their higher aggressiveness would allow them to
95 access to more resources or mates (Vandenberg et al., 2012). Individuals living under
96 laboratory conditions are usually fed ad libitum, which can potentially mask any reproductive
97 advantage of masculinisation due to IUP. In addition, results involving endocrinology and
98 behaviour can differ substantially between laboratory and field settings (Calisi & Bentley,
99 2009). Thus, empirical tests of IUP effects in the wild, where food availability is limiting, are
100 complementary to those performed under laboratory conditions. One study has explored IUP
101 effects under seminatural conditions by releasing laboratory-reared house mice onto a
102 highway island approximately 1ha in size (Zielinski et al., 1992). The only effect of IUP found
103 was a 45% larger home range size for 2M females than for 2F females (Zielinski et al., 1992).
104 However, the failure to detect a difference in survival rate or reproductive traits of females
105 from different IUP may have been due to the short duration of the 8-week experiment (vom

106 Saal et al., 1999). Unfortunately, the study of IUP effects outside laboratory is extremely
107 challenging since it requires a caesarean section in litters with more than 3 offspring to identify
108 the position of each foetus. In contrast to the IUP, litter sex composition (i.e. litter sex ratio or
109 co-twin sex) does not require a caesarean section and, consequently, is more easily
110 measurable. Thus, empirical studies testing for the effects of litter sex composition on
111 subsequent reproductive performance in captive animals, wild animals and humans have
112 accumulated in the last two decades.

113 Here, we review studies investigating the potential influence of litter sex composition
114 around the time of birth on subsequent reproductive performance in viviparous organisms.
115 After describing our literature search protocol, we discuss the results for each taxonomic
116 group. The variability across species in IUP effects (vom Saal et al., 1999) limits the relevance
117 of conducting an overall study across taxa.

118 **Literature review protocol**

119 Our search protocol consists of a backward-forward citation search. Backward citation search
120 aims at identifying references cited in an article, while forward citation search involves
121 identifying references that cite an article. As a first step, we searched backward and forward
122 citations of six highly relevant studies to our systematic review using ISI Web of Science on
123 August 15th 2022. These six classic papers correspond to a review on IUP effects (Ryan &
124 Vandenberg, 2002), a review on sex-specific sibling interactions in vertebrates (Uller, 2006),
125 a review on prenatal influences of hormones on reproductive life history strategies (Clark &
126 Galef, 1995), two early studies of IUP effects in mice (vom Saal & Bronson, 1978, 1980) and an
127 early study on long-term consequences of litter sex ratio on reproduction in swine (Drickamer
128 et al., 1997). After removing duplicated papers, we obtained 1033 studies. We then identified

129 76 studies potentially relevant to our synthesis after reading titles and abstracts. We then
130 inspected these 76 studies in detail and checked whether they still met our inclusion criteria
131 (see below). This allowed us to obtain 26 out of 44 studies displayed in table 1. We reiterated
132 the procedure (backward and forward citation searching, titles and abstracts screened, full
133 text papers screened) from these 26 studies until no further relevant studies could be
134 identified (three iterations; the last iteration on January, 15, 2023). The additional backward
135 citation searching was conducted manually by checking the reference list of the study. We
136 used Google Scholar instead of ISI Web of Science for the additional forward citation searching
137 because it can find much grey literature (Haddaway et al., 2015).

138 To be included in our review, the paper had to contain statistical information testing
139 the influence of litter sex composition on reproductive performance. These individuals belong
140 to the F0 generation. We included studies that measured litter sex composition at birth, or if
141 this was impossible, at weaning. For instance, in many social carnivores, females give birth to
142 their litter underground, and therefore measures of litter sizes at birth are not possible
143 (Russell et al., 2003). For twin studies, we included only those comparing opposite-sex and
144 same-sex twins. For instance, studies comparing the reproductive performance of twin
145 animals with non-twin animals (Dunn et al., 1979) were not included in our sample.

146 We classified the different traits of reproductive performance into 7 categories:
147 fecundity, fertility, post-natal survival of offspring, age at first reproduction, parental care,
148 interbirth interval and mating success. Fecundity is the biological ability of an individual to
149 conceive and have a live birth (Gardner et al., 2009). As such, fecundity can never be measured
150 directly and is only estimated from multiple indicators, such as the time to pregnancy,
151 probability of becoming parent, pregnancy success, stillbirth rate, ovulation rate, blastocyst

152 production and sperm concentration. Fertility is a productive term representing the number
153 of offspring an individual has (Gardner et al., 2009). We included the number of offspring per
154 litter or per unit time (e.g. year) in the category 'fertility'. The number of offspring was
155 measured either at birth or independence (weaning). Nevertheless, we included post-natal
156 survival of offspring (i.e. survival rate of F1 generation between birth and independence) as a
157 separate category because this is a measure specifically of offspring viability. The three traits
158 included in the category 'age at first reproduction' correspond to an actual reproduction (i.e.
159 age at first birth, age at first conception) or to a potential reproduction (i.e. age at sexual
160 maturity). Parental care mostly reflects offspring provisioning and protection in mammals
161 (Clutton-Brock, 1991). The most direct measures of food provisioning during the period of
162 lactation are milk production and composition (Landete-Castillejos et al., 2005) whereas
163 offspring mass at weaning is often used as indirect measure (Hamel et al., 2012). The category
164 'interbirth interval' includes time between births and number of litters per unit time. Finally,
165 we included dominance rank in animals and probability of marriage in human (von Rueden &
166 Jaeggi, 2016) in the category 'mating success'.

167 **Results of the literature survey**

168 *Characteristics of included studies*

169 Our search protocol allowed us to identify 44 studies of 28 species for a total of 176
170 relationships between litter sex composition during early development and reproductive
171 performance (Table 1). There was a strong bias towards research on mammals. Human is the
172 most represented species with 26% of relationships and 34% of studies. Farm animals and
173 captive primates were also well represented in the sample (61% of relationships). A striking
174 feature of the studies investigating the consequences of litter sex composition on

175 reproductive performance is their focus on females (81% of relationships). A female bias is
176 frequent in mammalian studies of life-history traits, in part because of the relative ease of
177 assigning maternity versus paternity (Archer et al., 2022). This bias is likely to have been
178 reinforced here by the economic importance of female productivity in farm animals because
179 there are no studies examining how litter sex composition influences male reproductive
180 performance in pigs, sheep, goats and rabbits (Table 1).

181 Litter sex composition was measured in different ways depending on litter size. When
182 there were two offspring per litter, studies have examined the effect of co-twin sex on
183 reproductive performance, as in sheep and human. When there were more than 3 offspring
184 per litter, studies have assessed the impact of litter sex ratio either by (i) comparing individuals
185 from same-sex vs mixed-sex litters, (ii) entering the proportion of males in the litter as a
186 continuous variable in the model, or (iii) converting the proportion of males in the litter to two
187 or more groups (Table S1). For the dependant variable, the 7 categories of reproductive
188 performance that we considered were represented in our sample but fecundity, fertility and
189 age at first reproduction represented over three quarters of the relationships (Table S1).

190 *Human (n = 15 studies)*

191 Several studies have tested the effects of co-twin sex on the same reproductive traits of
192 women from different populations. Inconsistent results are found for each of those
193 reproductive traits: the probability of giving birth, the number of offspring born, the age at
194 sexual maturity or the probability of marriage (Tables 1 and S1). Pre-industrial Finnish women
195 (born between 1734 and 1888) with a male co-twin in utero had reduced lifetime reproductive
196 success compared with women born with a co-twin female, as a result of a lower probability
197 of marriage as well as decreased fertility (Lummaa et al., 2007). A Norwegian study (Bütikofer

198 et al., 2019) including twins born between 1967 and 1978 has found that women born with
199 female co-twins had 11.7% lower probability of ever having married and 5.8% fewer children,
200 although it is unclear why these two variables were measured at age 32 rather than later. In
201 contrast, there were no differences in the number of children between women born of same-
202 or opposite-sex twin pairs in Australia, the Netherlands and USA during the 20th century
203 (Medland et al., 2008). A possible explanation for these divergent findings is spatio-temporal
204 variation in exposure to EDC. By altering the effects of endogenous hormones on
205 development, EDCs can make the direction and the magnitude of the relationships between
206 co-twin sex and reproductive performance hard to predict (Howdeshell et al., 1999; vom Saal,
207 2016). Although some EDCs are produced naturally by plants (phytoestrogens), the majority
208 are man-made compounds whose production started in the 1930s (Darbre, 2021). Thus, the
209 fitness consequences of co-twin sex have been relatively well studied in women potentially
210 exposed to EDCs (Figure 1). More studies are needed to confirm that in the past (e.g. before
211 the industrial revolution) women with male co-twins had reduced reproductive success.

212 It is also possible that the observed inconsistency between study results is, at least
213 partially, linked to another aspect of the in utero environment: nutrient competition (Box 1).
214 Males, being selected to grow faster, may be more successful in that competition, particularly
215 in poor environmental conditions (Oddie, 2000) such as encountered in pre-industrial
216 populations. Under this scenario, girls born with a sister co-twin should be heavier than those
217 born with a brother co-twin (James, 2002). In 15 developed countries, girls having a co-twin
218 brother were, at maximum (values depend on gestational age), 12 g heavier than those with
219 a co-twin sister (Jelenkovic et al., 2018), suggesting that this difference was due to a positive
220 effect of androgen on foetal growth (De Zegher et al., 1998) rather than nutrient competition

221 with male co-twin. However, the association between birth mass and co-twin sex remains to
222 be tested in less favourable conditions.

223 During their study examining the influence of co-twin sex on reproductive performance
224 in Norwegian women, Bütikofer et al. (2019) also measured long-term impacts on men. They
225 have found that men with a female co-twin had a lower probability of ever having married by
226 age 32 yr. However, this relationship was no longer significant for a subset of men with a
227 deceased co-twin within first year of life, suggesting that it reflects differences in post-birth
228 socialisation effects rather than hormonal influences in utero. In other populations, men had
229 a similar probability of marriage or produced similar number of children, irrespective of
230 whether they had a female or male co-twin (Lummaa et al., 2007; Steeno & Vlietinck, 1989;
231 Wyshak & White, 1969). This absence of effects is not really surprising. Indeed, while
232 numerous effects of IUP on behaviour and reproductive organs in male mice have been
233 attributed to differences in serum oestradiol during foetal life (vom Saal, 2016; vom Saal et
234 al., 1983), prenatal levels of oestriol and oestradiol do not appear to differ by foetal sex in
235 human (Inkster et al., 2021).

236 *Non-human primates (n = 6 studies)*

237 The reproductive consequences of litter sex composition have been analysed relatively
238 recently in captive callitrichines (marmosets, tamarins and lion tamarins). In this family of
239 primates, the early shared intrauterine vascularisation facilitates the transfer of signalling
240 molecules between co-twins, including hormones involved in sexual differentiation. However,
241 the majority of the relationships between increased presence of males in the litter and female
242 reproductive performance, when present, are positive (Table 1), i.e. in the opposite direction
243 of what we would expect based on testosterone transfer between male and female foetuses.

244 It has been suggested that mate competition between same-sex siblings limits the
245 reproductive performance of individuals born in same-sex litters compared to those born in
246 mixed-sex litters (McCoy et al., 2019). Although Perret (2021) found that male mouse lemurs
247 in same-sex litters were more competitive and produced more offspring than males born in
248 mixed-sex litters, she suggested that this pattern may result from social interactions after birth
249 rather than testosterone levels. Specifically, play fighting between brothers could play a major
250 role in the development of the skills or behaviours required for mating success (Perret, 2021).

251 *Ovine and caprine (n = 11 studies)*

252 Sheep play an important part in research on the freemartinism syndrome, which causes an
253 economic loss for farmers (Esteves et al., 2012). If the risk of freemartinism is too high, this
254 may counteract selection for large litter size in sheep populations. However, the incidence of
255 freemartins in ewes born with male siblings is only 3.15% (Marí & Casellas, 2018). This
256 contrasts with results in cattle where approximately 80-92% of females born co-twin to male
257 are freemartins (Padula, 2005). In addition, all XX/XY chimeric females born co-twin with a
258 male are freemartins and sterile in cattle (Eldridge & Blazak, 1977), whereas there are no
259 significant differences in the frequency of female chimeras between litters with different sex
260 ratios in sheep (Brace et al., 2008). These differences between sheep and cattle cannot be
261 explained by different structures of placentae. Indeed, the placenta in ruminants is multiple
262 and cotyledonic (Rousseau-Ralliard et al., 2020). A possible reason behind these disparities is
263 that sex differentiation occurs much earlier in sheep than in cattle (Iannuzzi et al., 2021).
264 Indeed, mixed-sex pregnancies can avoid the freemartinism phenomenon when placental
265 anastomoses fail to fuse or when fusion occurs after the critical period of sexual differentiation
266 (Marí & Casellas, 2018).

267 In domestic sheep, blastocyst production rates and embryonic survival were increased
268 in ewes born with a female co-twin compared with those born with a male co-twin, but the
269 co-twin sex or litter sex ratio did not affect the number of offspring born or weaned (Table 1).
270 In contrast, in feral Soay sheep, female born with a male co-twin had lower number of lambs
271 born over their entire life compared with those born with a female co-twin (Korsten et al.,
272 2009). Those differences in lifetime reproductive success were mainly due to higher first-year
273 mortality of females born with a male co-twin. In addition, female Soays born with a male co-
274 twin had reduced birth mass relative to those born with a female co-twin (Korsten et al., 2009),
275 which is the opposite of what we would expect based on the hypothesis of testosterone-
276 induced masculinisation. It is likely that nutrient competition in utero contributes to the
277 observed pattern in the population of Soay sheep consequent to low resource availability (a
278 function of population density and weather).

279 Only two studies have been conducted in goats. The coexistence of a female with one
280 or two males in utero did not alter the fecundity, fertility and age at first birth of adult does
281 (Mellado et al., 2005). However, female goats that developed in utero with a male co-twin
282 produced more milk, but with lower fat percentage, than those that developed in utero with
283 a female co-twin (Abecia et al., 2020). The effect of milk volume diluting macronutrients has
284 been observed in other species (Chalupa et al., 2000; Hinde, 2009). In sheep, no effect of the
285 co-twin sex on female milk production was detected (Avdi & Driancourt, 1997); however this
286 study was limited to the first lactation and the results were in the same direction as those in
287 goats. This should encourage researchers to investigate whether male-specific foetal
288 hormones cause changes in the mammary glands of their female litter mates. In particular,
289 such investigations could be insightful to evaluate the potential benefits of considering sex
290 composition of the litter in the breeding programs used in dairy species.

291 *Porcine (n = 4 studies)*

292 The four studies in pigs found at least one statistically significant correlation between litter
293 sex ratio and one reproductive trait (Table 1). It is certainly possible, but has not been
294 examined, that the complex counter-current uterine blood flow system in swine females leads
295 to an effect on maternal hormone levels that is different from what occurs in rodents, that
296 have a very different uterine vasculature. Thus, a foetal pig may be more sensitive to the
297 hormonal contribution of every foetus belonging to the litter than that of adjacent foetuses
298 (Ryan & Vandenberg, 2002). In support of this idea, age at puberty of female swine was
299 related to litter sex ratio but not IUP (Lamberson et al., 1988; Rohde Parfet et al., 1990). Some
300 results are more difficult to interpret. For instance, it is unclear why the direction of the
301 relationship between the sex-ratio in the litter in which the mother was born and piglet
302 mortality seen in Polish Landrace pigs is the opposite of that seen in Polish Large White pigs
303 (Rekiel et al., 2012).

304 *Rodents (n = 7 studies)*

305 In two different populations of Alpine marmots (Dupont et al., 2015; Hackländer & Arnold,
306 2012), females from male-biased litters were more likely to become dominant than those
307 from female-biased litters. As in many other cooperative breeders, competition for
308 reproductive opportunities is particularly intense in this species, where reproduction within
309 social groups is monopolised by a single dominant female. While studies carried out in
310 laboratory rodents have shown a negative influence of masculinisation on female
311 reproductive performance, the opposite happens in Alpine marmots. A possible reason for
312 this discrepancy is that the relationships between aggressiveness and mating success are
313 variable between species. In rodents used in laboratory experiments, masculinised females

314 being more aggressive are less receptive to males and less preferred by them (Ryan &
315 Vandenberg, 2002). In some wild animals, the benefits of aggressiveness are probably higher
316 than its costs. In support of this idea, female Alpine marmots from male-biased litters are
317 more likely to become dominant by fighting, defeating and expelling the previous dominant
318 (Hackländer & Arnold, 2012). If masculinised females are also more aggressive towards male
319 intruders, masculinisation in Alpine marmots might also reduce the risk of infanticide by males
320 (Coulon et al., 1995). Trade-offs between development of traits increasing competitiveness,
321 like fighting ability, and fitness components may occur as in other taxa. For instance, in rustic
322 breeds of Swiss domestic cattle, the selection for fighting ability has led to reductions both in
323 milk production and fecundity (Sartori et al., 2015). However, in female alpine marmots, there
324 was no evidence for effects of litter sex ratio at weaning on the number of young per litter or
325 during the entire period of dominance as an adult (Hackländer & Arnold, 2012).

326 The effects of litter sex ratio on female reproduction performance have been studied
327 in another marmot species. In the yellow-bellied marmot, the probability of reproduction in a
328 given year was independent of litter sex ratio (Monclús et al., 2014). The yellow-bellied
329 marmot is primarily polygynous with low reproductive skew (reproduction is shared rather
330 equally) among females of a social group compared all other social marmot species, including
331 the Alpine marmot (Allainé, 2000). Litter sex composition may have a stronger influence on
332 competitive ability and the acquisition of dominance in species with high levels of
333 reproductive skew (i.e. when reproduction is monopolised by a single individual of each sex
334 per social group).

335 In *Peromyscus* rodents, males reared in litters with more brothers develop larger testes
336 as adults and this difference in testis size is associated with increased fertility (Fisher et al.,

337 2018). As discussed by Fisher et al. (2018), it was unknown whether differences in testis size
338 among *Peromyscus* males are due to differential investment in sperm production by the males
339 themselves and/or passive exposure to higher levels of testosterone in utero. However, a
340 study on wild house mice found support for the former but not latter possibility (Lavoie et al.,
341 2019), suggesting that growing males allocate more in sperm production under a perceived
342 risk of male-male competition.

343 *Reptiles (n = 1 study)*

344 Only one study in the common lizard showed that females from male-biased clutches grew
345 faster and matured earlier, but had reduced fecundity (Uller et al., 2004). In addition, male
346 common lizards from male-biased clutches were more likely to successfully reproduce at age
347 one than males from female-biased clutches (Uller et al., 2004). To our knowledge, no other
348 study in non-mammalian viviparous species has investigated whether reproductive
349 performance of females and males may depend on the sex ratio of their litter of origin, but
350 there are two studies of snakes which have examined the consequence of litter sex ratios on
351 sex-specific morphological traits (Osypka & Arnold, 2000; Weatherhead et al., 2006). Thus,
352 there is potential for more work to be done to further understand if and how prenatal sex
353 ratio influence future reproduction in reptiles.

354 **Attempts to explain differences between studies based on IUP vs litter sex composition**

355 Relationships between litter sex composition and reproductive performance appear more
356 discordant and complex than those between IUP and reproduction. For twin studies, IUP is
357 equivalent to litter sex composition. Even if sex hormones transfer between twins, it is
358 possible that the level of in utero hormonal exposure from a single opposite sex co-twin is not
359 high enough to induce reproductive consequences. However, laboratory studies show that

360 fetuses situated between a single male and a female fetus (1MF fetuses) have
361 intermediate concentrations of testosterone and oestradiol, and in adulthood are
362 intermediate between 2M and 2F individuals of the same sex in many morphological,
363 physiological and behavioural traits (vom Saal, 2016; vom Saal et al., 1999).

364 In studies that examined the influence of litter sex ratio on future reproductive
365 performance, litter sex ratio was generally used as a proxy of prenatal exposure to
366 androgens/oestrogens while it was unknown whether fetuses developed in a close proximity
367 to opposite-sex fetuses, or not. If not, they might have been less affected by androgens and
368 oestrogens than 2M and 2F individuals. Litters containing many males may increase the
369 concentrations of testosterone in the mother's circulation and, in turn, increase blood
370 concentration of this hormone in the entire litter. However, there was no evidence for this
371 possibility in mice (vom Saal & Bronson, 1980).

372 Outside the laboratory, there are many factors that can mask relationships between
373 litter sex composition and reproductive performance (e.g. exposure to EDCs in human
374 studies). Conversely, some relationships between litter sex composition and reproductive
375 performance could arise via pathways that have nothing to do with prenatal sex hormones
376 (Box 1).

377 **Conclusions**

378 Back to our initial questions, our review of the literature in viviparous organisms (n = 44 studies
379 in 28 species) shows that evidence for an association between litter sex composition around
380 the time of birth and adult reproductive performance is currently conflicting. Almost all
381 studies were on mammal species, principally humans and farm animals. In humans, the
382 absence of long-term effects of co-twin sex on fecundity and fertility in the majority of

383 contemporary populations is reassuring given the increase in the frequency of twin births in
384 nearly all countries (Monden et al., 2021). In future studies, it will be important to differentiate
385 the effects of sex hormones in utero from the other pathways (Box 1). These different
386 pathways may explain why relationships between litter sex composition and reproductive
387 performance are variable even between populations within the same species. In sum, the
388 effects of litter sex composition around the time of birth on subsequent reproduction are less
389 clear compared with IUP effects in laboratory rodents.

390 **Declaration of interest, Funding, Contributions and Acknowledgements**

391 We declare that there is no conflict of interest that could be perceived as prejudicing the
392 impartiality of the research reported. This research did not receive any specific grant from any
393 funding agency in the public, commercial or not-for-profit sector. MD conceived the study.
394 MD, VR and FD performed literature search and wrote the paper. We thank two anonymous
395 reviewers for their helpful comments.

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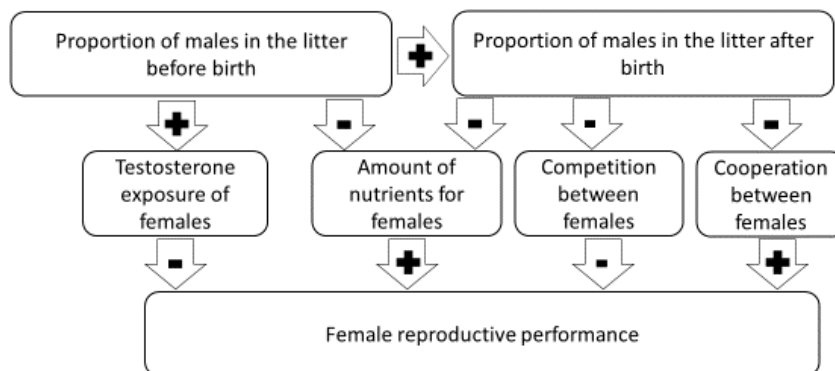
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716

717

718 **BOX**

719 BOX 1. Causal mechanisms of how correlations between litter sex composition and
720 subsequent reproductive performance of females can arise. The sign of each arrow represents
721 the sign of the causal relationship associated.



722

723 Our understanding of how litter sex composition around the time of birth influences
724 reproductive performance later in life is limited. In females, these effects are commonly
725 attributed to testosterone-induced masculinisation in utero but there are plausible alternative
726 explanations.

727 The first alternative involves nutritional competition between males and females
728 during gestation, particularly in sexually dimorphic species. Sex differences in energy intake
729 and growth of juveniles are common in sexually size-dimorphic species (Clutton-Brock, 1991).
730 Sexually size dimorphism in most mammals is biased in favour of males and this difference can
731 be evident soon after conception (Bukowski et al., 2007). Males, growing faster, may be more
732 successful in the competition for nutrients during prenatal development. Thus, females from

733 male-biased litters may be nutritionally disadvantaged compared with females from female-
734 biased litters. This can lead to long-term consequences. For instance, variation in prenatal
735 growth can influence subsequent growth and age-specific size which, in turn, influence
736 reproductive performance (Lummaa & Clutton-Brock, 2002).

737 Sex-specific nutritional disadvantage after birth may also contribute to the differences
738 in reproductive performance of individuals in relation to their co-twin sex or litter sex ratio.
739 This disadvantage may be a consequence of sex differences in competitive ability or of
740 contrasts in parental strategy, or of a combination of both. Lactation, in particular, may be an
741 important mechanism underlying such effects. In mammals, in general the energetic costs of
742 gestation are small in comparison with those of lactation (Clutton-Brock, 1991). If daughters
743 are less favoured than sons by mothers, through milk quantity (Landete-Castillejos et al.,
744 2005), being reared with more males may be detrimental to females. Under this scenario,
745 prenatal litter sex composition is not the causal factor, it simply determines postnatal litter
746 sex composition.

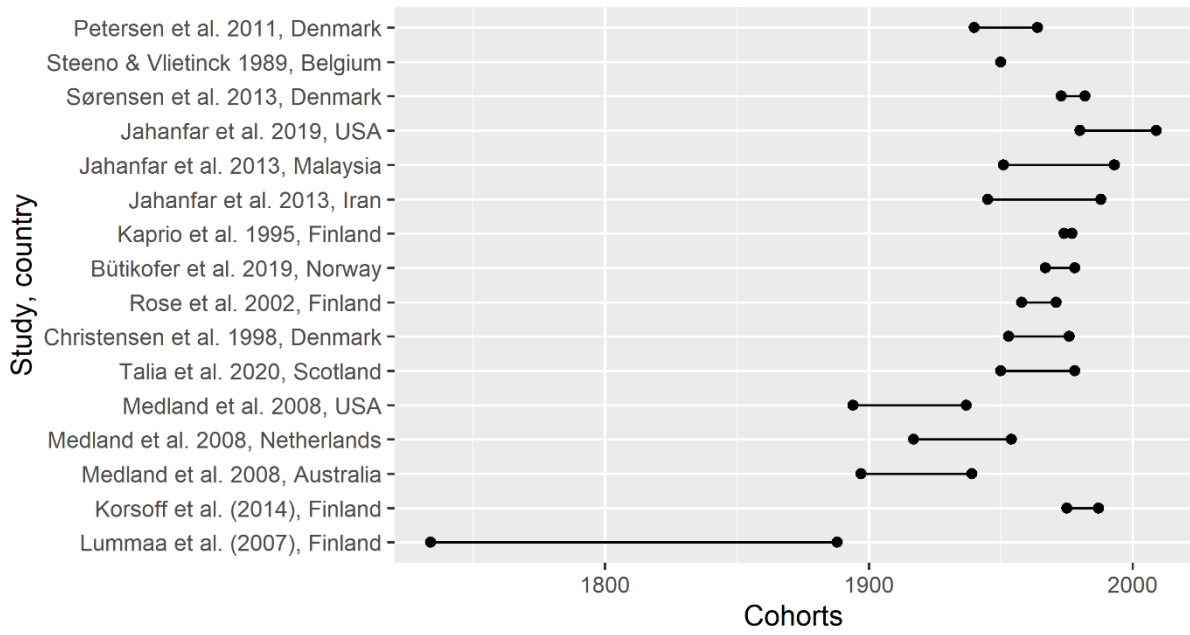
747 Post-natal litter sex composition may also influence reproductive performance via
748 competition or cooperation between same-sex siblings for mates. In African lions (*Panthera*
749 *leo*), kin-related males form coalitions to take over and then defend a group of females (Packer
750 et al., 1991). Conversely, mate competition between same-sex siblings limits the reproductive
751 performance of individuals born in same-sex litters compared to those born in mixed-sex
752 litters (McCoy et al., 2019). The relationship between the proportion of males in the litter and
753 reproductive performance of females is expected to be positive under the hypothesis of
754 reproductive competition, but negative under the hypothesis of testosterone-induced
755 masculinisation, nutritional competition or reproductive cooperation.

756 **Figure legend**

757

758 Figure 1. Birth year range for human studies in which relationships between co-twin sex and
759 reproductive performance have been examined. Cohorts were reported for each study listed
760 in table 1, except two (Loehlin & Martin 1998, Wysak & White 1969).

761 **Figure 1**



762

763

764 **Table legends**

765 Table 1. Summary of the 44 studies testing whether litter sex composition influenced
766 subsequent reproductive performance of females (F) and males (M). For each species and sex,
767 we reported the number of positive, negative or null (i.e. p -value > 0.05) relationships
768 between increased presence of males in the litter and subsequent reproductive performance.
769 The detailed data set, with one row per relationship, is provided in supplementary material
770 (Table S1). The superscript numbers indicate the following used taxonomic groups: 1, human;
771 2, non-human primates; 3, ovine and caprine; 4, porcine; 5, rodents; 6, reptile.

772 Table S1. Studies testing whether litter sex composition influenced subsequent reproductive
773 performance of females (F) and males (M). We report whether the relationship between
774 increased presence of males in the litter and subsequent reproductive performance was
775 positive (+), negative (-) or non-statistically significant (0, i.e. p -value > 0.05). This does not
776 always match the direction of the correlation between column "D" and "E". For instance, in
777 common marmoset, females born with a co-twin male lost more foetuses than females born
778 with a co-twin female (Rutherford et al., 2014), so the reported effect (column "F") is negative.

779

