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Maternal age, paternal age, and litter size interact to affect the offspring sex ratio of German Shepherd dogs

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A B S T R A C T

Several factors influence mammalian sex ratio. In this study, the effects of parental age and litter size on the offspring sex ratio in dogs were evaluated in 602 puppies born from 101 German Shepherd litters from a breeding facility. The data was obtained from recordings of the kennel and Studbook from 1996 to 2016. A linear mixed-model analysis was performed using dam age, sire age and litter size as predictors of the percentage of males in litter. Dam age and litter size has direct effect on sex ratio. Dam age * sire age and dam age * litter size interactions are significant. When evaluating dam age by sire age, the expected male percentage in the litter is higher for old dams mated with young sires and young dams mated with old sires. Smaller percentage of males is expected for young dams mated with young sires and old dams mated with old sires. When evaluating dam age by litter size, the expected percentage of males in the litter is higher for young dams delivering big litters and old dams delivering small litters. Smaller percentage of males is expected for young dams delivering small litters and old dams delivering big litters. Dam aging increased proportion of males in litters. We concluded that offspring sex ratio is determined partially by the dam age, sire age and litter size interactions in German Shepherd dogs.

1. Introduction

Sexual determination is an intriguing question [1]. The theoretical 1:1 sex ratio at the time of conception [2] does not correspond to that observed at birth in mammals [3], and there are several possibilities to explain this difference [4,5]. The bias in the sex ratio may result from mother and/or father influence acting alone or in combination [5], and such combination might be antagonistic or complementary, increasing the frequency of one or the other sex at birth, or even having a neutral effect [6].

Several factors that may bias the sex ratio in mammals have been previously studied (reviewed by Cameron [7], Rosenfeld and Roberts [8], Gutierrez-Adan et al. [9], Aurich and Schneider [10] and Booksmythe et al. [11]), and despite of contradictory reports, parental age is considered a relevant factor [12–20]. However, for polytocous species this effect is controversial (reviewed by Cameron [7] and Rosenfeld and Roberts [8]), and in domestic dogs, dam age and parity order do not seem to influence the offspring sex ratio [21].

Trivers and Willard [22] proposed that older mothers tend to generate more females than males because of the high mortality of Ysptz and/or of the XY embryos within the female genital tract, and that this embryo survival control probably occurs through the

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glucose metabolism of the mother (Trivers-Willard hypothesis - TWH). Differences in male and female embryos are observed even before phenotypic dimorphism [23,24], resulting in metabolic differences during pre-implantational period observed in many mammalian species (reviewed by Gutierrez-Adan et al. [9]). The male embryo needs higher glucose levels when compared to female embryo. Therefore, in low glucose concentrations male embryos are more susceptible to mortality than females [7,25]. Including some polytocous species [26], the TWH was supported in 34% of the original studies and not supported in 8.5%, whereas the data from the remaining 57.5% had non-significant results [7]. Polygynous and sexually dimorphic species are good candidates to be supported by TWH [27] so the litter size should be considered a relevant variable in the determination of sex ratio of the litter in polytocous species [28].

Most studies on this topic have been performed on wildlife (reviewed by Cameron [7] and Aurich and Schneider [10]), but domestic species may have a different pattern due to the effects of domestication [29–31]. For instance, Lord et al. [30] described several changes in reproductive traits due to domestication of dogs (*Canis lupus familiaris*), which occurred at least 14,000 years ago [32]. Gavrilovic et al. [21] did not find correlations between sex ratio and several reproductive traits in domestic dogs, including age of the bitch. Studying golden hamsters (*Mesocricetus auratus*), Huck et al. [13] demonstrated that sex-selective embryo death alters simultaneously sex ratio and size of the litter, as the reduction of sex specific embryos also reduces the size of the offspring. However, the current data on these parameters are still controversial for wild [27,33] and domestic [34–37] mammalian species.

There are limited studies on factors that affect offspring sex ratio in dogs. Tedor and Reif [38] observed that several breeds, including German Shepherd, had higher sex ratio than expected, although others had smaller sex ratio than expected, suggesting that breed may affect sex ratio in dogs. Recently, Gharagozlou et al. [39] observed a higher proportion of males in the litter of Siberian Husky bitches (62.4%) compared to German Shepherd bitches (50.0%), which is in agreement to the Tedor and Reif's [38] hypothesis of breed influence on litter sex ratio. Gharagozlou et al. [39] also observed a higher proportion of males in the litter of bitches that received fish oil (64.0%) compared to those that received palm oil (46.3%) supplementation for 30 days before conception. To the best of our knowledge, the influence of litter size on the offspring sex ratio has not been investigated in domestic dogs.

The knowledge of factors influencing sex ratio in domestic dogs may help breeders, technicians and scientists to understand more about consequences of breeding practices on reproductive process of this species. The aim of this study was to evaluate the influence of parental age and litter size on sex ratio at birth in German Shepherd dogs.

2. Material and methods

2.1. Animals

This study was based on a retrospective analysis of 602 puppies that belonged to 101 litters derived from mating of 45 dams and 37 sires in a private breeding facility of German Shepherd dogs from 1996 to 2016 (Table 1).

2.2. Breeding management

All dams were housed at the kennel, but sires were either from the same or from other facilities. Data of litter composition and birth date were obtained from the recordings of the kennel, and all animals, including stillbirths, were considered in this study, independently of their registration in the official Studbook. Parental dates of birth were obtained from the database of the Brazilian Club of German Shepherd (www.sbcpa.com.br).

Table 1
Dams (n = 45) and sires (n = 37) and their respective number of litters in a German Shepherd facility (1996–2016).

Number of dams	Number of litters	Number of sires	Number of litters
21	1	26	1
9	2	7	2
6	3	1	3
5	4	2	10
3	5	1	12
1	9	1	26

Adult animals were housed individually. Dams were mated naturally according to the interests of the kennel, although artificial insemination was performed in some estrous cycles, not included in our analysis. Whelping occurred in maternity pens, where the bitches and their litters stayed for at least 40 days until weaning. The animals received commercial food two times a day and water *ad libitum*. Sanitary measures included periodical control of endo- and ectoparasites, vaccines against the common diseases of dogs, and disinfection of the premises. Except for the postpartum dams, the dogs were exercised at least once a day.

2.3. Statistical analysis

In order to verify the effect of dam age, sire age, and litter size on the sex ratio (number of males divided by number of females born), a saturated mixed model was fitted considering all the former effects and their interactions terms as fixed. Dam and sire were included in the model as random effects due to multiple offspring, accounting for dependence between observations from litters for the same individuals. For random effects it was assumed a Gaussian distribution that were checked using diagnostic plots. We used the percentage of males in the litter instead of sex ratio of the litter in analyses because in litters composed of only one gender the sex ratio could not be calculated (i.e., *n* males/0 females or 0 males/*n* females). A backward selection model methodology was performed aiming to obtain the most parsimonious model that explained sex ratio. The criterion used to exclude explanatory variables were the significance of the effects. Models generated in each step of backward selection method were compared by their AIC (Akaike Information Criterion) and the final model was chosen by the lowest AIC value. Likelihood ratio tests after adjust models to be compared by means of maximum likelihood method was also used to guarantee the best model choice. The final model to explain sex ratio was then fitted by restricted maximum likelihood including the fixed effects of the dam age, sire age, litter size, dam age * sire age interaction, dam age * litter size interaction, and random effects of dam and sire. A pseudo-r^2 was obtained following Kagawa and Schielzeth [40].

Fixed effects were estimated and surface plots were drawn to better understanding the modulation of dam age effect on sex ratio by sire age and litter size levels. Predictions of sex ratio for the combination of dam and sire of 2 and 8 years old and litter sizes of 2 and 10 puppies were obtained in order to highlight interaction effects. Prediction accuracy of the final model was evaluated as the Pearson correlation between predicted values and realized values.

All analyses were performed using the R statistical software version 3.5.3 [41]. Mixed models were fitted using lmer function of the lme4 package [42]. The considered significance level for all analyses was 0.05.

3. Results

The average age of the 45 bitches was 4.7 ± 2.1 (mean ± standard deviation) years, with a minimum of 1.8 and a maximum of 8.7, whereas the average age of the 37 sires was 5.6 ± 2.1, with a minimum of 1.6 and a maximum of 11.0. The average litter size was 6.0 ± 2.4 (mean ± standard deviation) with a minimum of 1 and a maximum of 11 puppies and the sex ratio was 1.03 (305 males and 297 females).

Effects of the dam age ($P < 0.01$), litter size ($P < 0.01$), dam age * sire age interaction ($P = 0.03$) and dam age * litter size interaction ($P = 0.01$) were significant on sex ratio of offspring. The effect of the dam age had the greater magnitude comparing other as the percentage of males is expected to increase in 15% with one year increasing in the age of the dam. Moreover, this effect is modulated by the sire age and litter size (Table 2).

Regarding dam age * sire age interaction, higher percentage of males is expected for old dams when mated with young sires, as well as for young dams mated by old sires; and smaller percentage of males were predicted for young dams when mated with young sires, as well as for old dams when mated with old sires. When evaluating dam age * litter size interaction, higher percentage of males were predicted for young dams delivering big litters and old dams delivering small litters; and smaller percentage of males were predicted for young dams delivering small litters and old dams delivering big litters (Fig. 1).

The highest predicted percentage of males was 81.6% for litters of 2 puppies (small litter) born from 8.0 year dam (old) and 2.0 year sire (young). The smallest estimated percentage of males was 19.7% for litters of 2 puppies (small litter) born from 2.0 year dam (young) and 2.0 year sire (young). Only two-way interactions affected the sex ratio, since the percentage of males is expected to increase for young dams when the litter size is bigger, regardless the sire age. On the contrary, the relative frequency of males in litter is expected to decrease for old dams when give birth to bigger litters independently of sire age. Additionally, the sex ratio increased when young dam is bred with older sires, regardless litter size. Also, percentage of born males decreased for old females bred with young sires independently of the litter size (Table 3).

4. Discussion

Our data demonstrated that the age of the dam influenced sex ratio in domestic dogs interacting with litter size and sire age. Sire age was a non-significant predictor of the litter sex ratio, but it had a relevant interaction with dam age. To the best of our knowledge, these findings have not been previously observed in domestic dogs.

The dogs included in this study were representative of German Shepherds in regard to sex ratio and litter size. The whole offspring percentage of males in our German Shepherd population was very similar to that observed for several breeds [38], including German Shepherds [38,39]. The average litter size of our dog group was lower than those observed for large breeds [43]; but considering German Shepherds, it was either similar [44] or lower [38,45] than those previously reported.

For golden hamsters under laboratory condition, another polytocous mammalian species, Huck et al. [13] hypothesized that a sex-selective embryo death, which depends on the dam age, resulted in reduction of litter size concomitantly to the sex ratio bias. Our results are compatible to the Huck et al. [13] hypothesis, supporting the notion of a similar trend in dogs.

Although controversial [7], most of the studies that evaluated the effects of maternal age on offspring sex ratio showed that old mothers tend to have more females than males in their offspring [12–17,19]. The effect of litter size on sex ratio is also controversial. In wild dogs (*Lycaon pictus*) [33] and domestic cats (*Felis catus*) [34], litter size did not influenced sex ratio. However, high proportion of females have been observed in small litters of wild pigs (*Sus scrofa scrofa*) [27], the opposite observed in domestic pigs (*Sus scrofa domesticus*), with high proportion of females in large litters in a study [36]. Although, in domestic pigs litter size was considered irrelevant in other studies [35,37].

Although the effects of maternal age and litter size remains controversial, our study demonstrated that bitch age and litter size interacts to determine offspring sex ratio, and cannot be analyzed independently. However, the studies of this interactive effect are scarce in literature. It was considered irrelevant in wild pigs [27]; but older golden hamster mothers had high proportion of females in small litters, but not in large litters [13]. Our analyses showed that old bitches with small litters had high proportion of males in their litter, contrary to that observed in golden hamsters [13]; but young bitches with small litters had high proportion of females, which have not been observed in golden hamsters [13]. A sex-selective mortality of embryos has been proposed for hamsters [13], which may also be the case for dogs.

We have also observed that despite the fact that the effect of sire age on offspring sex ratio was not significant ($P = 0.08$), sire age

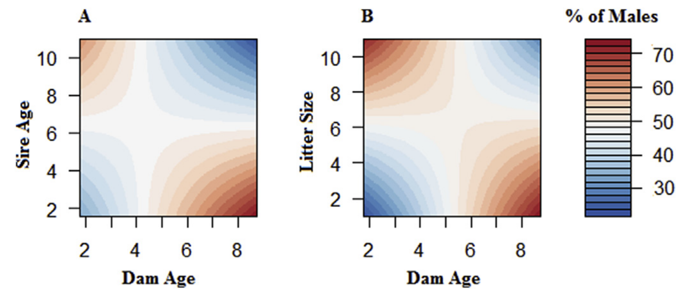

Fig. 1. Graphical representation of the percentage of males in the offspring born in a German Shepherd facility (1996–2016). A) Representation of Dam Age * Sire Age interaction; B) Representation of Dam Age * Litter Size interaction.

Table 2
Estimates for effects of dam age, sire age, litter size and interactions on the percentage of males in the litter in a German Shepherd facility (1996–2016).

Explanatory variables	Estimate (standard error)	P value
Intercept	−0.2484 (0.2241)	0.27
Dam age	0.1523 (0.0416)	<0.01
Sire age	0.0475 (0.0265)	0.08
Litter size	0.0721 (0.0268)	<0.01
Dam age * Sire age	−0.0115 (0.0054)	0.03
Dam age * Litter size	−0.0130 (0.0500)	0.01

Total pseudo-r2 = 0.1453; AIC = 7.7 (AIC from saturated model = 11.2).

Table 3
Percentage of males predictions for 8 simulated litters considering only the fixed effects as predictors derived from data from a German Shepherd facility (1996–2016).

Simulated litter	Dam age (y)	Sire age (y)	Litter size	% of males
1	2.0	2.0	2	19.7285
2	2.0	2.0	10	56.5157
3	2.0	8.0	2	34.4351
4	2.0	8.0	10	71.2223
5	8.0	2.0	2	81.6155
6	8.0	2.0	10	55.6859
7	8.0	8.0	2	54.7853
8	8.0	8.0	10	28.8557

Accuracy = 0.36.

interacted with the dam age affecting offspring sex ratio. Interaction effect of mother and father characteristics has been previously reported. Most of the hypotheses for offspring sex ratio determination in mammals attributes to the mother the ability of facilitating or inhibiting Xsptz or Ysptz fertilization of the oocytes [5,46]. In addition to sex-selective embryo death, the skew of offspring sex ratio may also result from different intrinsic ability of Xsptz or Ysptz to reach and fertilize the oocytes; or it may be due to the female sex-elective effects on spermatozoa influencing their arrival to the fertilization site and/or their ability to fertilize the oocyte [5]. The female genital environment may influence the spermatozoa progression differently according to its sex chromosome content (reviewed by Rosenfeld [47]) and, indeed, it has been demonstrated that Xsptz or Ysptz evoke different gene expression profiles in porcine oviductal cells [48].

We observed that parents with low age gap resulted in lower sex ratio in the litter, but higher age gap results in higher sex ratio, independently of who is the older or the younger parent. The age gap is considered a factor that affects sex ratio in man [14,20,49], golden lion tamarins (*Leontopithecus rosalia*) [17] and horses (*Equus caballus*) [19]. Our results are in good agreement with these previous studies, which demonstrated that lower age gap resulted in more females, and higher age gap resulted in more males in the offspring. The influence of mother and father in the offspring sex ratio can be antagonistic or complementary, resulting in different possibilities of offspring sex ratio depending on the power and direction of the mother and father effects [6,50]. Our results demonstrated an antagonistic influence of father and mother, although more powerful for mother than father, as the sire effect alone was not significant.

Conflicting parental influences on sex ratio may cause unfitness to the TWH [50], and it is the case here, as the parental effects were antagonistic. Our results also did not fit the TWH because the influence of the old dam was for more sons, and the hypothesis states that raising females is more advantageous for dams with low resources [22,25,51,52], for example old mothers which affect sex ratio by a sex-selective mortality of XY-embryos at early stages of the gestation [22]. We also found that the lower the litter size, the higher the litter sex ratio, which is in accordance with higher mortality of XX-embryos, diverging from the TWH.

The Canidae family has several reproductive features that are expressed in wild species, but not in the domestic dog, including the typical cooperative breeding behavior of wild polytocous species [30,48]. Females of wild Canidae species usually are assisted by helpers, which are non-breeding members of the group who protect and feed the puppies [30,53]. In African wild dogs, male siblings are best helpers than females. According to Creel et al. [33], this is probably the reason for primiparous bitches deliver more males than females, as they will need helpers more than the multiparous bitches for the next litter. Our results of more females in small litters of young bitches did not corroborate those findings described by Creel et a [33]. However, the cooperative behavior of wild Canidae was strongly influenced by domestication [31], since in a dog facility feeding the dam and puppies as well as pup-rearing are provided by man, making helpers unnecessary [30,54,55]. Senior domestic bitches do not need male siblings to help raising their puppies. Considering that skew of sex ratio may be adaptive in domestic dogs, why would senior bitches need more males in their offspring? Conversely, why would young bitches need more females in their offspring? If adaptive, this skew in sex ratio linked to dam age may be derived from an unknown sex-linked needs of dogs under domestic conditions that differs from those in the wild.

Male age did not affect litter sex ratio in our study, although many evidences suggest an active role of males on sex ratio determination [5,6], which may be associated with selective production, ejaculation or fertilizing ability of different sex chromosome bearing spermatozoa [56–58]. Edwards and Cameron [6] revised the literature and showed variations in Ysptz:Xsptz ratio in semen of several species associated to several conditions, including age, which was negatively correlated to Ysptz percentage in semen of men [18,59], but not in a captive population of pigmy hippopotamus (*Choeropsis liberiensis*) where father age significantly increased offspring sex ratio [60]. The absence of effect of sire age on litter sex ratio observed in this study not necessarily indicated that Ysptz:Xsptz ratio was not affected by the age of the sire, since the female genital tract may differently affect Ysptz or Xsptz. In horses, the male effect upon sex ratio was only observed after analyses of thousands gestations, but not observed in a small number of gestations [19], and it can be the case for our population of dogs, with an insufficient number of observations to detect the sire age effect, although the sire age affected sex ratio when interacting with the dam effect.

There is obvious need for additional studies in order to elucidate mechanisms of sex ratio determination in dogs. Although significant, the fitness of field data to the statistical model is small enough to consider the trends here observed biologically relevant in a dog breeding facility or other small population of dogs, and the combined effect of dam age and litter size, and of dam age and sire age, maybe will only be observed in a large population of dogs. This is the first report of parental age effect on offspring sex ratio in dogs, and we strongly encourage other studies to confirm our observation and elucidate mechanisms involved.

In conclusion, dam age, sire age and litter size are important factors to be considered when litter sex ratio of German Shepherd dogs is a subject of concern. It is expected that older dams that give birth to smaller litters or that mated with older sires have higher percentage of males in offspring. Also, young dams that give birth to smaller litters or that mated young sires are expected to give birth of higher percentage of females in offspring. Considering the lack of studies about sex ratio determination in domestic dogs, our results expand knowledge of the reproductive biology of dogs supporting breeding decisions. Nevertheless, the biological mechanisms involved in sex ratio modulation is still not completely elucidated in dogs.

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References

[1] Mittwoch U. Sex determination in mythology and history. Arq Bras Endocrinol Metabol 2005;49:7–13. https://doi.org/10.1590/S0004-27302005000100003.
[2] Fisher RA. The genetical theory of natural selection. New York: Dover Publications; 1958.
[3] Hamilton WD. Extraordinary sex ratios. Science 1967;156:477–88. https://doi.org/10.1126/science.156.3774.477.
[4] Berry DP, Kearney JF, Roche JR. Evidence of genetic and maternal effects on secondary sex ratio in cattle. Theriogenology 2011;75:1039–44. https://doi.org/10.1016/j.theriogenology.2010.11.011.
[5] Cameron EZ, Edwards AM, Parsley LM. Developmental sexual dimorphism and the evolution of mechanisms for adjustment of sex ratios in mammals. Ann N Y Acad Sci 2017;1389:147–63. https://doi.org/10.1111/nyas.13288.
[6] Edwards AM, Cameron EZ. Forgotten fathers: paternal influences on mammalian sex allocation. Trends Ecol Evol 2014;29:158–64. https://doi.org/10.1016/j.tree.2013.12.003.
[7] Cameron EZ. Facultative adjustment of mammalian sex ratios in support of the Trivers-Willard hypothesis: evidence for a mechanism. Proc Roy Soc Lond B 2004;271:1723–8. https://doi.org/10.1098/rspb.2004.2773.
[8] Rosenfeld CS, Roberts RM. Maternal diet and other factors affecting offspring

sex ratio: a review. Biol Reprod 2004;71:1063–70. https://doi.org/10.1095/biolreprod.104.030890.
[9] Gutierrez-Adan A, Perez-Crespo M, Fernandez-Gonzalez R, Ramirez MA, MoreiraP Pintado B, Lonergan P, Rizos D. Developmental consequences of sexual dimorphism during pre-implantation embryonic development. Reprod Dom Anim Suppl 2006;2:54–62. https://doi.org/10.1111/j.1439-0531.2006.00769.x.
[10] Aurich C, Schneider J. Sex determination in horses – current status and future perspectives. Anim Reprod Sci 2014;146:34–41. https://doi.org/10.1016/j.anireprosci.2014.01.014.
[11] Booksmythe I, Mautz B, Davis J, Nakagawa S, Jennions MD. Facultative adjustment of the offspring sex ratio and male attractiveness: a systematic review and meta-analysis. Biol Rev 2015;198:625–37. https://doi.org/10.1111/brv.12220.
[12] Caughley G. Offspring sex ratio and age of parents. J Reprod Fertil 1971;25:145–7. https://doi.org/10.1530/jrf.0.0250145.
[13] Huck UW, Pratt NC, Labov JB, Lisk RD. Effects of age and parity on litter size and offspring sex ratio in golden hamsters (*Mesocricetus auratus*). J Reprod Fertil 1988;83:209–14. https://doi.org/10.1530/jrf.0.0830209.
[14] Manning JT, Anderton RH, Shutt M. Parental age gap skews child sex ratio. Nature 1997;389:344. https://doi.org/10.1038/38647.
[15] Jacobsen R, Møller H, Mouritsen A. Natural variation in the human sex ratio. Hum Reprod 1999;14:3120–5. https://doi.org/10.1093/humrep/14.12.3120.
[16] Nicolich MJ, Huebner WW, Schnatter AR. Influence of parental and biological factors on the male birth fraction in the United States: an analysis of birth certificate data from 1964 through 1988. Fertil Steril 2000;73:487–92. https://doi.org/10.1016/S0015-0282(99)00576-2.
[17] Bayfield A, Young RJ. The effect of parental age gap on the sex ratio of Golden Lion tamarins born in zoos. Folia Primatol (Basel) 2002;73:214–6. 65424.
[18] Stone BA, Alex A, Werlin LB, Marrs RP. Age thresholds for changes in semen parameters in men. Fertil Steril 2013;100:952–8. https://doi.org/10.1016/j.fertnstert.2013.05.046.
[19] Santos MM, Maia LL, Nobre DM, Oliveira Neto JF, Garcia TR, Lage MC, de Melo MI, Viana WS, Palhares MS, da Silva Filho JM, Santos RL, Valle GR. Sex ratio of equine offspring is affected by the ages of the mare and stallion. Theriogenology 2015;84:1238–45. https://doi.org/10.1016/j.theriogenology.2015.07.001.
[20] Matsuo K, Ushioda N, Udoff LC. Parental aging synergistically decreases offspring sex ratio. J Obstet Gynaecol Res 2009;35:164–8. https://doi.org/10.1111/j.1447-0756.2008.00836.x.
[21] Gavrilovic BB, Andersson K, Linde Forsberg C. Reproductive patterns in the domestic dog - a retrospective study of the Drever breed. Theriogenology 2008;70:783–94. https://doi.org/10.1016/j.theriogenology.2008.04.051.
[22] Trivers RL, Willard DE. Natural selection of parental ability to vary the sex ratio of offspring. Science 1973;179:90–2. https://doi.org/10.1126/science.179.4068.90.
[23] Kobayashi S, Isotani A, Mise N, Yamamoto M, Fujihara Y, Kaseda K, Nakanishi T, Ikawa M, Hamada H, Abe K, Okabe M. Comparison of gene expression in male and female mouse blastocysts revealed imprinting of the X-linked gene, Rhox5/Pem, at preimplantation stages. Curr Biol 2006;16:166–72. https://doi.org/10.1016/j.cub.2005.11.071.
[24] Bermejo-Alvarez P, Rizos D, Rath D, Lonergan P, Gutierrez-Adan A. Sex determines the expression level of one third of the actively expressed genes in bovine blastocysts. Proc Natl Acad Sci USA 2010;107:3394–9. https://doi.org/10.1073/pnas.0913843107.
[25] Cameron EZ, Lemons PR, Bateman PW, Benett NC. Experimental alterations of litter sex ratios in a mammal. Proc Roy Soc Lond B 2008;275:323–7. https://doi.org/10.1098/rspb.2007.1401.
[26] Szenczi P, Bánszegi O, Groó Z, Altbäcker V. Anogenital distance and condition as predictors of litter sex ratio in two mouse species: a study of the house mouse (*Mus musculus*) and mound-building mouse (*Mus spicilegus*). PLoS One 2013;8:e74066. https://doi.org/10.1371/journal.pone.0074066.
[27] Servanty SS, Gaillard J-M, Allainé D, Brandt S, Baubet E. Litter size and fetal sex ratio adjustment in a highly polytocous species: the wild boar. Behav Ecol 2007;18:427–32. https://doi.org/10.1093/beheco/arl099.
[28] Willians GC. The question of adaptive sex ratio in outcrossed vertebrates. Proc Roy Soc Lond B 1979;205:567–80. https://doi.org/10.1098/rspb.1979.0085.
[29] Chelini MO, Souza NL, Otta E. Breeding system can affect the offspring sex ratio in Syrian hamsters. Lab Anim 2011;45:55–7. https://doi.org/10.1258/la.2010.010099.
[30] Lord K, Feinstein M, Smith B, Coppinger R. Variation in reproductive traits of members of the genus Canis with special attention to the domestic dog (*Canis familiaris*). Behav Process 2013;92:131–42. https://doi.org/10.1016/j.beproc.2012.10.009.
[31] Marshall-Pescini S, Virányi Z, Range F. The effect of domestication on inhibitory control: wolves and dogs compared. PLoS One 2015;10:e0118469. https://doi.org/10.1371/journal.pone.0118469.
[32] Vila C, Savolainen P, Maldonado JE, Amorin IR, Rice JE, Honeycutt RL, Crandall KA, Lundeberg J, Wayne RK. Multiple and ancient origins of the domestic dog. Science 1997;276:1687–9. https://doi.org/10.1126/science.276.5319.1687.
[33] Creel S, Creel NM, Monfort SL. Birth order, estrogens and sex-ratio adaptation in African wild dogs (*Lycaon pictus*). Anim Reprod Sci 1998;53:315–20. https://doi.org/10.1016/S0378-4320(98)00121-3.
[34] Johnstone I. Reproductive patterns of pedigree cats. Aust Vet J 1987;64:197–200. https://doi.org/10.1111/j.1751-0813.1987.tb15180.x.
[35] Soede NM, Nissen AK, Kemp B. Timing of insemination relative to ovulation in pigs: effects on sex ratio of offspring. Theriogenology 2000;53:1003–11. https://doi.org/10.1016/S0093-691X(00)00246-6.
[36] Górecki MT. Sex ratio in litters of domestic pigs (*Sus scrofa f. domestica* Linnaeus, 1758). Biol Lett 2003;40:111–8.
[37] Alfonso L. Use of meta-analysis to combine candidate gene association studies: application to study the relationship between the ESR PvuII polymorphism and sow litter size. Genet Sel Evol 2005;37:417–35. https://doi.org/10.1051/gse:2005009.
[38] Tedor JB, Reif JS. Natal patterns among registered dogs in the United States. J Am Vet Med Assoc 1978;172:1179–85.
[39] Gharagozlou F, Youssefi R, Akbarinejad V. Effects of diets supplemented by fish oil on sex ratio of pups in bitch. Vet Res Forum 2016;7:105–10.
[40] Nakagawa S, Schielzeth H. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods Ecol Evol 2013;4. https://doi.org/10.1111/j.2041-210x.2012.00261.x. 33-142.
[41] R Core Team. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2014.
[42] Bates D, Maechler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. J Stat Softw 2015;67:1–48. https://doi.org/10.18637/jss.v067.i01.
[43] Borge KS, Tonnessen R, Nodtvedt A, Indrebo A. Litter size at birth in purebred dogs – a retrospective study of 224 breeds. Theriogenology 2011;75:911–9. https://doi.org/10.1016/j.theriogenology.2010.10.034.
[44] Mutembei HM, Mutiga ER, Tsuma VT. An epidemiological survey demonstrating decline in reproductive efficiency with age and non-seasonality of reproductive parameters in German shepherd bitches in Kenya. J S Afr Vet Assoc 2002;73:36–7.
[45] Šichtař J, Dokoupilová A, Vostrý L, Rajmon R, Jílek F. Factors affecting reproductive efficiency in German Shepherd bitches producing litters for Police of the Czech Republic. Czech J Anim Sci 2016;61:578–85. https://doi.org/10.17221/89/2015-CJAS.
[46] Hardy ICW. Possible factors influencing vertebrate sex ratios: an introductory overview. Appl Anim Behav Sci 1997;51:217–41. https://doi.org/10.1016/S0168-1591(96)01106-9.
[47] Rosenfeld CS. Periconceptional influences on offspring sex ratio and placental responses. Reprod Fertil Dev 2012;24:45–58. https://doi.org/10.1071/RD11906.
[48] Almiñana C, Caballero I, Heath PR, Maleki-Dizaji S, Parrilla I, Cuello C, Gil MA, Vazquez JL, Vazquez JM, Roca J, Martinez EA, Holt WV, Fazeli A. The battle of the sexes starts in the oviduct: modulation of oviductal transcriptome by X and Y-bearing spermatozoa. BMC Genet 2014;15:293. https://doi.org/10.1186/1471-2164-15-293.
[49] Manning JT, Anderton RH. Age difference between husbands and wives as a predictor of rank, sex of first child, and asymmetry of daughters. Evol Hum Behav 1998;19:99–110. https://doi.org/10.1016/S1090-5138(98)00017-8.
[50] Douhard M. The role of fathers in mammalian sex allocation. Mamm Rev. 2017;48:67–74. https://doi.org/10.1111/mam.12112.
[51] McGinley MA. The adaptive value of male biased sex ratios among stressed animals. Am Nat 1984;124:597–9. https://doi.org/10.1086/284299.
[52] Byers JA, Moodie JD. Sex-specific maternal investment in pronghorn, and the question of a limit on differential provisioning in ungulates. Behav Ecol Sociobiol 1990;26:157–64. https://doi.org/10.1007/BF00172082.
[53] Lukas D, Clutton-Brock T. Life histories and the evolution of cooperative breeding in mammals. Proc Roy Soc Lond B 2012;279:4065–70. https://doi.org/10.1098/rspb.2012.1433.
[54] Bradshaw JSW. The evolutionary basis for the feeding behavior of domestic dogs (*Canis familiaris*) and cats (*Felis catus*). J Nutr 2006;136:1927S–31S.
[55] Silva-Rodriguez EA, Sieving KE. Influence of care of domestic carnivores on their predation on vertebrates. Conserv Biol 2011;25:808–15. https://doi.org/10.1111/j.1523-1739.2011.01690.
[56] Pérez-Crespo M, Pintado B, Gutiérrez-Adán A. Scrotal heat stress effects on sperm viability, sperm DNA integrity, and the offspring sex ratio in mice. Mol Reprod Dev 2008;75:40–7. https://doi.org/10.1002/mrd.20759.
[57] Eisenberg ML, Murthy L, Hwang K, Lamb DJ, Lipshultz LI. Sperm counts and sperm sex ratio in male infertility patients. Asian J Androl 2012;14:683–6. https://doi.org/10.1038/aja.2012.58.
[58] Edwards AM, Cameron EZ. Cryptic male choice: experimental evidence of sperm sex ratio and seminal fluid adjustment in relation to coital rate. Reprod Fertil Dev 2016. https://doi.org/10.1071/RD16123.
[59] Sartorelli EMP, Mazzucatto LF, Pina-Neto JM. Effect of paternal age on human sperm chromosomes. Fertil Steril 2001;76:1119–23. https://doi.org/10.1016/S0015-0282(01)02894-1.
[60] Saragusty J, Hermes R, Hofer H, Bouts T, Göritz F, Hildebrandt TB. Male pigmy hippopotamus influence offspring sex ratio. Nat Commun 2012;3:697. https://doi.org/10.1038/ncomms1700.