RESEARCH NOTE

Short-term effect of ovariohysterectomy on urine serotonin, cortisol, testosterone and progesterone in bitches

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Abstract

Objective: This study aimed to investigate the short-term effect of ovariohysterectomy on urine levels of serotonin and its relation to levels of cortisol, testosterone and progesterone in female dogs. Seven bitches were studied before surgical ovariohysterectomy and then once a week during 4 weeks. Spontaneously voided urine samples were collected and concentration ratios of hormone/creatinine in urine were analysed.

Results: The bitches had significantly lower levels of cortisol, testosterone, and progesterone 1 week after ovariohysterectomy compared with before and the levels stayed low throughout the study ($P \leq 0.05$). Interestingly, serotonin levels tended to increase 4 weeks after surgery ($P = 0.08$). A positive correlation between cortisol and progesterone was found before and after surgery. After surgery, serotonin was positively correlated with cortisol and progesterone ($P \leq 0.05$).

Keywords: Bitches, Canine, Cortisol, Gonadectomy, Neuter, Progesterone, Serotonin, Spay, Testosterone, Urinary hormones

Introduction

Dog owners may have their dogs neutered to reduce specific behaviours or to resolve persisting behaviour problems [1]. Higher cortisol levels have been associated with different types of stress situations [2] as well as aggression [3] and fear [4, 5]. Lower cortisol levels in gonadectomized dogs compared to intact dogs have been found in saliva [6], as well as in serum [7]. However, other studies found no such differences in circulating cortisol concentrations [8, 9]. Serotonin levels in dogs have been inversely correlated with aggression [3, 10–12] and impulsivity [13]. Expression of serotonin transporter (5-HTT) in peripheral blood mononuclear cells was positively correlated with plasma cortisol in infant rhesus macaques [14]. Lower levels of plasma/serum testosterone have been reported in castrated male dogs compared to intact dogs [8, 15, 16]. Aggressiveness was found to be related to testosterone level in male dogs [17], male Ethiopian wolves [18] and boars [19]. GnRH agonist slow-release implants, that inhibit testosterone production, play an important role in the reduction of aggressiveness in male dogs [17] and even might have impact on behaviour of female dogs [20]. It is known that in bitches, the ovaries are sites of testosterone production [14, 21]. Recently, in a study on women, the authors suggested that an aggressive response to provocation was related to high levels of testosterone in saliva [22]. Plasma progesterone levels have been reported to be lower in neutered than intact dogs of both genders [8]. To our knowledge there is only one publication concerning changes of urinary concentration of serotonin in gonadectomized dogs, without information about the time between surgery and experiment [23]. In rats an increased function of

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hypothalamic serotonin-1A receptor was seen after ovariectomy [24]. Thus, there is no previous data about the effect of gonadectomy on serotonin levels in dogs. The objective of this study was therefore to investigate the effect of short-term ovariohysterectomy on urine levels of serotonin and its relation to levels of cortisol, testosterone and progesterone in female dogs. The hypothesis was that gonadectomy affects the serotonin concentrations and if a possible correlation with other hormones exists.

Main text
Methods
Animals
Seven privately owned bitches (mean 3.6 years) of different breeds were recruited at three animal veterinary clinics (see Additional file 1: Table S1). The study only included dogs whose owners had already decided to have their dogs neutered before accepting to participate in this study.

The bitches were in late diestrus or early anestrus (mean 4.1 months after heat) and none had previously been chemically neutered nor given pharmaceuticals to delay or disrupt heat (see Additional file 1: Table S1). Surgical procedures were done at three clinics in Uppsala, Sweden. All bitches were subjected to ovariohysterectomy through ventral midline incision. No dog had fever or depressed general condition or was on regular medication before or after gonadectomy except for postoperative analgesic prescription from their respective veterinarian. Moreover, one dog was treated with gastroprotective sucralfate for 7 days after surgery (see Additional file 1: Table S1). Participation in this study did not affect the ordinary treatment of the dogs before, during, and after surgery except for urine sampling. Urine sampling was approved by the Animal Ethics Committee in Uppsala (C287/12); the use of privately owned animals by the Swedish Board of Agriculture (Dnr 31-11700/12).

Urine samples
Spontaneously voided urine samples were collected on six occasions by the owner: the evening before surgery, the morning of surgery, and every seventh morning for the next 4 weeks. Sample 1 was collected during the previous evening dog walk and all other samples were collected during the first morning dog walk, prior to the dog's first meal. The urine was transferred into dark coloured tubes containing 3.2 M HCl (150 μL/mL), to improve sustainability by acidification to optimize the serotonin analysis. Urine was also transferred to empty test tubes for analysis of creatinine, cortisol, testosterone and progesterone. Samples were stored at -20 °C during the experiment, and at -70 °C when the last sample was collected until analysis.

Analyses
Creatinine was quantified by Arbor Assays Urinary Creatinine Detection Kit (Michigan, USA). MDV was determined to 15.1 μmol/L and the intraassay CV was < 10% between 53 and 1768 μmol/L. The analyses of each respective hormone were performed on 2 different days, with samples from one half of the group analysed on each day. All hormones were analysed by ELISA according to manual guidelines. Cortisol was quantified by IBL International Urine Cortisol ELISA (Hamburg, Germany), serotonin by IBL International Serotonin ELISA (Hamburg, Germany), testosterone by Testosterone Arbor Assays Nordic Biosite, and progesterone by Progesterone Arbor Assay Nordic Biosite (Stockholm, Sweden). Due to one missing sample, six samples were analysed for serotonin from the morning before surgery (sample 2). Analytical data are presented in Additional file 2: Table S2.

Statistics
Physiological data were analysed with SAS Software 2008 (Statistical Analysis Systems, 9.2, SAS Institute, Cary, NC, USA) and presented as mean values for each sample (SEM) $\pm$ Standard Errors (SE). ANOVA (Mixed Procedure) were used to study whether samples from bitches differed significantly. Samples taken the morning before surgery were chosen as controls, and designated as the reference to which all samples collected post-surgery were compared. The statistical model included the fix effect of sample and the random effect of individual. The Pearson correlation coefficient was applied to investigate correlations between the hormones before (sample 1–2) and after (sample 3–6) ovariohysterectomy. The significance level was set at $P \leq 0.05$.

Results
Serotonin
Before and 1 week after ovariohysterectomy, the concentration ratio of serotonin/creatinine in urine varied between 22.8 and 24.2 nmol/mmol. Afterward, an increase of concentration ratio of serotonin/creatinine was observed. Four weeks after surgery the concentration ratio was 33.7 nmol/mmol, which tended to be higher ($P = 0.08$) than the morning before (Fig. 1).

Cortisol
The urinary cortisol/creatinine ratio in the morning sample was significantly higher than in the evening sample before surgery (12.1 vs. 8.9 nmol/mmol, respectively, $P \leq 0.05$). Ovariohysterectomy resulted in a significant decrease of urinary cortisol in the
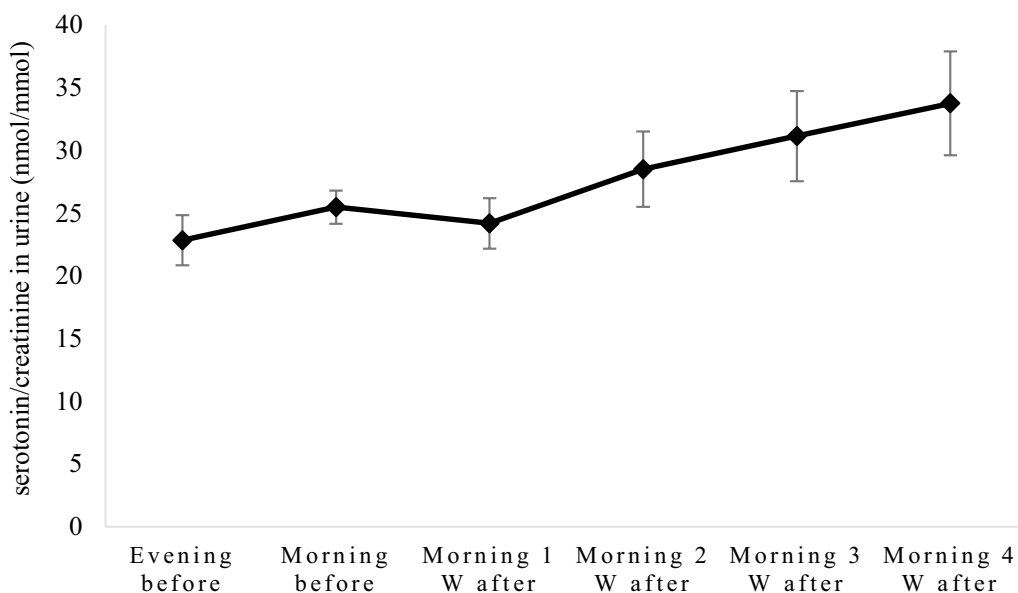
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Fig. 1 Serotonin/creatinine ratios (mean value ± standard error) in seven bitches before and after ovariohysterectomy. W = weeks. The morning sample before surgery included only six bitches due to problems with collecting. The serotonin levels did not differ significantly between the morning before ovariohysterectomy and the other samples. Four weeks after surgery the levels tended to be higher than that before (P = 0.08)

morning samples within 1 week after surgery (from 12.1 to 8.44 nmol/mmol, P ≤ 0.05). The low concentrations of cortisol were observed during the entire experimental period and were not different from the concentration in the evening sample before ovariohysterectomy (Fig. 2).

Testosterone and progesterone

Before ovariohysterectomy, the urinary testosterone/creatinine ratio was 1.8 pmol/mmol. After surgery, a dramatic significant decrease in testosterone concentration was seen achieving 0.57 pmol/mmol 4 weeks later. Evening and morning samples did not differ before surgery (Fig. 3a). The urinary progesterone/creatinine ratio significantly decreased after surgery
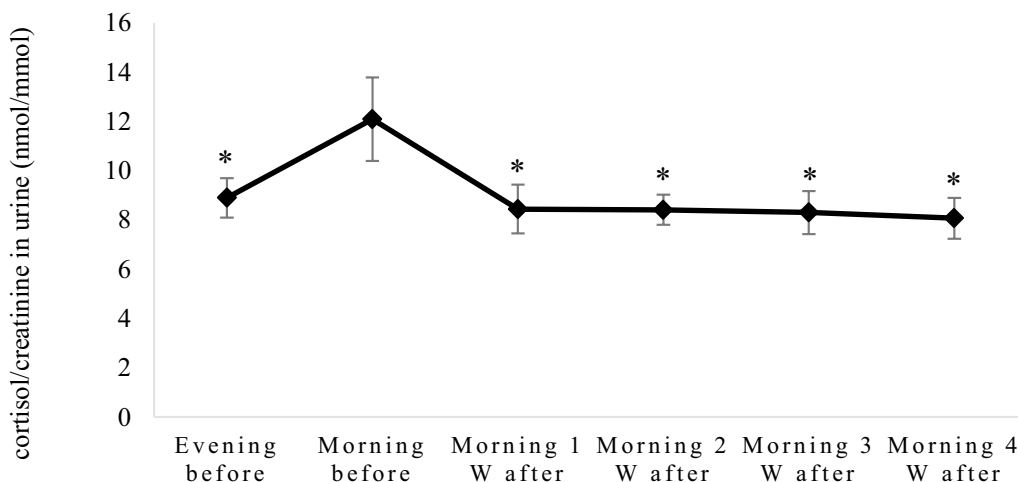

Fig. 2 Cortisol/creatinine ratios (mean value ± standard error) in seven bitches before and after ovariohysterectomy. W = weeks. The cortisol level was higher the morning before ovariohysterectomy compared with all samples after ovariohysterectomy and the evening before. *Significantly different from morning sample before ovariohysterectomy. P ≤ 0.05
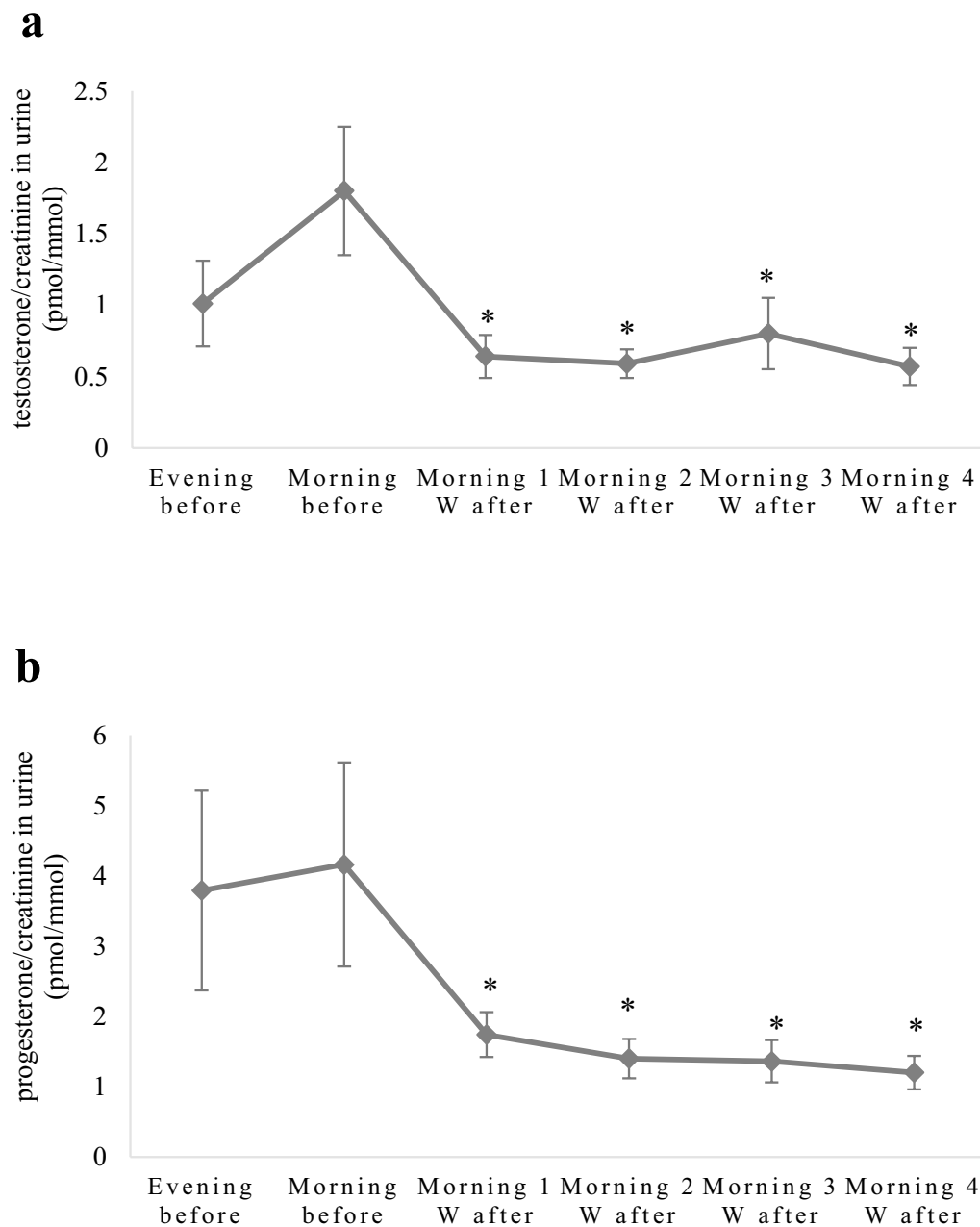
a

b

Fig. 3 **a** Testosterone/creatinine ratios (mean value ± standard error) in seven bitches before and after ovariohysterectomy. W = weeks. The testosterone levels decreased after ovariohysterectomy and stayed low in all samples. Evening and morning ratios before ovariohysterectomy did not differ. *Significantly different from morning sample before ovariohysterectomy. P ≤ 0.05. **b** Progesterone/creatinine ratios (mean value ± standard error) in seven bitches before and after ovariohysterectomy. W = weeks. The progesterone level decreased after ovariohysterectomy and stayed low in all samples. Evening and morning ratios before ovariohysterectomy did not differ. *Significantly different from morning sample before ovariohysterectomy. P ≤ 0.05

from 4.16 pmol/mmol and stayed low between 1.2 and 1.74 pmol/mmol throughout the experimental period. Evening and morning samples did not differ. Individual levels diverged more before than after surgery (Fig. 3b).

Correlations between hormones

There was a positive correlation between cortisol and progesterone before and after surgery (r = 0.72, P = 0.004 and r = 0.67, P = 0.0001, respectively). After surgery

serotonin was positively correlated with cortisol ($r = 0.44$, $P = 0.02$) and progesterone ($r = 0.38$, $P = 0.05$). See Additional file 3: Table S3.

Discussion

To our knowledge, this is the first study about the short-term effect of ovariohysterectomy on serotonin levels in bitches. In the present study, the urinary serotonin/creatinine ratio in bitches tended to be higher 4 weeks after surgery. In addition, serotonin was positively correlated with both cortisol and progesterone but not with testosterone after ovariohysterectomy. In male rats, castration increased serotonin-1A messenger RNA content in the cortex, hypothalamus, hippocampus, and amygdala [25], which may indicate an inverse relationship between testosterone and serotonin. A positive correlation between post-stress cortisol measures and serotonin 1A receptor ligand binding levels across multiple cortical and subcortical regions was reported in humans [26]. It was suggested that both serotonin and cortisol are associated in sex-differentiated responses to stress and possibly to anxiety, as they both rise in response to stress, especially in women [27]. The positive correlation between serotonin and cortisol found in our study was in agreement with previous report that serotonin affect the secretion of CRH and ACTH at the hypothalamic, pituitary gland level, and possibly also at the adrenal gland level [28]. In addition, serotonin, microinjected to paraventricular nucleus of conscious rats, activated the CRH synthesis, which resulted in increased plasma ACTH [29]. A plausible explanation for the positive correlation between serotonin and progesterone is that progesterone is secreted from adrenal glands after ovariohysterectomy. The effect of ACTH stimulation in ovarioectomized bitches have earlier been reported [8]. Recently, interbreed variation of serum serotonin in healthy dogs was reported [30]. The authors also found that males had higher levels of serotonin in serum than bitches [30]. Urine serotonin/creatinine ratio in our ovariohysterectomized bitches varied approximately between 20 and 40 nmol/mmol and was somewhat lower than in gonadectomized dogs of both genders (i.e. between 50 and 90 nmol/mmol) [23]. It is difficult to explain the reason for the higher serotonin levels in ovariectomized bitches. The present data may be important for power analysis in future studies and therefore serves as important reference material. Further studies are required to evaluate why and what effect this may have on the individual dog.

The bitches had lower cortisol levels 1 week after surgery than the morning before, and these levels remained low. Reportedly, the circadian rhythm of cortisol secretion results in high concentrations in the morning and low in the evening [31, 32]. Furthermore, the circadian rhythm in plasma cortisol has been reported to be disrupted in old dogs and not yet developed in puppies [31]. However, other authors found no circadian rhythm in cortisol in male dogs [33, 34] and bitches [34]. In agreement with our findings, saliva cortisol levels have been found to be lower in spayed bitches than in intact bitches [6] and lower serum cortisol levels have also been found in bitches 7–15 days after spaying than before [7]. Other studies found that plasma cortisol levels did not differ but they did not study the same individuals before and after gonadectomy [8, 9].

In our study, we found that ovariohysterectomy in bitches dramatically decreased urinary testosterone. This is in contrast to a study where plasma testosterone did not differ between intact and spayed females [8]. However, our results agreed with other studies that found basal plasma testosterone levels significantly higher before than after gonadectomy in female dogs [15]. These authors also reported that GnRH administration before gonadectomy caused significantly increased plasma testosterone levels in bitches. This observation agrees with another report that suggested that the ovaries may be sites of testosterone production in female dogs [21]. Thus, our findings provide further evidence that testosterone originates mainly from the ovaries in bitches.

In the present study, the progesterone levels decreased significantly 1 week after gonadectomy and stayed low throughout the experiment. Plasma progesterone levels have been reported to be lower in neutered dogs of both genders [8].

The relation between hormones and behaviour is complex and was outside stipulated aims of the study. However, measurements of serotonin, cortisol, testosterone and progesterone in urine collected by a non-invasive process may be useful for understanding of both female and male dogs' behaviour after gonadectomy.

Limitations

The number of dogs was relatively small and the bitches were of different breeds and in different phases of estrous cycle.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13104-021-05680-y.

Additional file 1: Table S1. Participating dogs.

Additional file 2: Table S2. Hormonal analyses.

Additional file 3: Table S3. Hormonal correlations.

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Authors' contributions

EHS, EL, and OVH participated in the design of the study. EL was responsible for the contact with dog owners and clinics and collected data and urine samples. AM evaluated the ELISA-analyses and statistics. EHS, EL, AM and OVH evaluated the results. EHS and EL drafted the manuscript and AM and OVH revised it. All authors read and approved the final manuscript.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study only included dogs whose owners had already decided to have their dogs neutered before accepting to participate in this study. Dog owners were informed about the study design and signed their approval. Participation in this study did not affect the ordinary treatment of the dogs before, during, and after surgery except for the spontaneously voided urine sampling. Urine sampling was approved by the Animal Ethics Committee in Uppsala (C287/12); the use of privately owned animals by the Swedish Board of Agriculture (Dnr 31-11700/12).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Andersson A, Linde-Forsberg C. Castration and progestagen treatment of male dogs, Part 2. EJCAP. 2002;12:178–85.
2. Beerda B, Schilder MBH, Van Hooff J, De Vries HW, Mol JA. Behavioural and hormonal indicators of enduring environmental stress in dogs. Anim Welf. 2000;9:49–62.
3. Rosado B, Garcia-Belenguer S, Leon M, Chacon G, Villegas A, Palacio J. Blood concentrations of serotonin, cortisol and dehydroepiandrosterone in aggressive dogs. Appl Anim Behav Sci. 2010;123:124–30.
4. Dreschel NA, Granger DA. Physiological and behavioural reactivity to stress in thunderstorm-phobic dogs and their caregivers. Appl Anim Behav Sci. 2005;95:153–68.
5. Hydbring-Sandberg E, Von Walter LW, Höglund K, Svartberg K, Swenson L, Forkman B. Physiological reactions to fear provocation in dogs. J Endocrinol. 2004;180:439–48.
6. Sandri M, Colussi A, Perrota MG, Stefanon B. Salivary cortisol concentration in healthy dogs is affected by size, sex, and housing context. J Vet Behav Clin Appl Res. 2015;10:302–6.
7. Srithunyarat T, Höglund OV, Hagman R, Olsson U, Stridsberg M, Lagerstedt A-S, et al. Catestatin, vasostatin, cortisol, temperature, heart rate, respiratory rate, scores of the short form of the Glasgow composite measure pain scale and visual analog scale for stress and pain behaviour in dogs before and after ovariohysterectomy. BMC Res Notes. 2016;9:381.
8. Frank LA, Rohrbach BW, Bailey EM, West JR, Oliver JW. Steroid hormone concentration profiles in healthy intact and neutered dogs before and after cosyntropin administration. Domest Anim Endocrinol. 2003;24:43–57.
9. Mongillo P, Prana E, Gabai G, Bertotto D, Marinelli L. Effect of age and sex on plasma cortisol and dehydroepiandrosterone concentrations in the dog (*Canis familiaris*). Res Vet Sci. 2014;96:33–8.
10. Cakiroglu D, Meral Y, Sancak AA, Cifti G. Relationship between the serum concentrations of serotonin and lipids and aggression in dogs. Vet Rec. 2007;161:59–61.
11. Leon M, Rosado B, Garcia-Belenguer S, Chacon G, Villegas A, Palacio J. Assessment of serotonin in serum, plasma, and platelets of aggressive dogs. J Vet Behav Clin Appl Res. 2012;7:348–52.
12. Reisner IR, Mann JJ, Stanley M, Huang YY, Houpt KA. Comparison of cerebrospinal fluid monoamine metabolite levels in dominant-aggressive and non-aggressive dogs. Brain Res. 1996;1(714):57–64. https://doi.org/10.1016/0006-8993(95)01464-0.
13. Wright HF, Mills DS, Pollux PMJ. Behavioural and physiological correlates of impulsivity in the domestic dog (*Canis familiaris*). Physiol Behav. 2012;105:676–82.
14. Kinnally EL, Lyons LA, Abel K, Mendoza S, Capitanio JP. Effects of early experience and genotype on serotonin transporter regulation in infant rhesus macaques. Genes Brain Behav. 2008;7:481–6.
15. De Gier J, Buijtels JJCWM, Albers-Wolthers CHJ, Oei CHY, Kooistra HS, Okkens AC. Effects of gonadotropin-releasing hormone administration on the pituitary-gonadal axis in male and female dogs before and after gonadectomy. Theriogenology. 2012;77:967–78.
16. Garde E, Perez GE, Vanderstichel R, Dalla Villa PF, Serpell JA. Effects of surgical and chemical sterilization on the behaviour of free-roaming male dogs in Puerto Natales. Chile Prev Vet Med. 2016;123:106–20.
17. Goericke-Pesch S, Wilhelm E, Ludwig C, Desmoulins PO, Driancourt MA, Hoffmann B. Evaluation of clinical efficacy of Gonazon implants in the treatment of reproductive pathologies, behavioural problems, and suppression of reproductive functions in the male dog. Theriognelogy. 2010;73:920–6.
18. Van Kesteren F, Sillerio-Zubiri C, Millar R, Argaw K, Macdonald DW, Paris M. Sex, stress and social status: patterns in fecal testosterone and glucocorticoid metabolites in male Ethiopian wolves. Gen Comp Endocrinol. 2012;179:30–7.
19. Parois SP, Prunier A, Mercat MJ, Merlot E, Larzul C. Genetic relationships between measures of sexual development, boar taint, health, and aggressiveness in pigs. J Anim Sci. 2015;93:3749–58.
20. Goericke-Pesch S. Long-term effects of GnRH agonists on fertility and behaviour. Reprod Domest Anim. 2017;52:336–47. https://doi.org/10.1111/rda.12898.
21. Olson PN, Bowen RA, Behrendt MD, Olson JD, Nett TM. Concentrations of testosterone in canine serum during late anestrus, proestrus, estrus, and early diestrus. Am J Vet Res. 1984;45:145–8.
22. Probst F, Golle J, Lory V, Lobmaier JS. Reactive aggression tracks within-participant in women's salivary testosterone. Aggr Behav. 2018;44:362–71.
23. Alberghina D, Piccionea G, Pumilia G, Gioè M, Rizzo M, Raffo P, Panzera M. Daily fluctuation of urine serotonin and cortisol in healthy shelter dogs and influence of intraspecific social exposure. Physiol Behav. 2019;206:1–6.
24. Raap DK, DonCarlos LL, Garcia F, Zhang Y, Muma NA, Battaglia G, Van de Kar LD. Ovariectomy-induced increases in hypothalamic serotonin-1A receptor function in rats are prevented by estradiol. Neuro Endocrinol. 2002;76(6):348–56. https://doi.org/10.1159/000067582.
25. Zhang L, Ma W, Barker JL, Rubinow OR. Sex differences in expression of serotonin receptors (subtypes 1A and 2A) in rat brain: a possible role of testosterone. Neuroscience. 1999;94:251–9.
26. Steinberg LJ, Rubin-Falcone H, Galfalvy HC, Kaufman J, Miller JM, Sublette ME, Cooper TB, Min E, Keilp JG, Stanley BH, Oquendo MA, Ogden RT, Mann JJ. Cortisol stress response and in vivo PET imaging of human brain serotonin. Int J Neuropsychopharmacol. 2019;22:329–38.

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27. Maes M, Vandewoude M, Schotte C, Maes L, Martin M, Blockx P. Sex-linked differences in cortisol, ACTH and prolactin responses to 5-hydroxy-tryptophan in healthy controls and minor and major depressed patients. Acta Psychiatr Scand. 1989;80:584–90.

28. Jørgensen HS. Studies on the neuroendocrine role of serotonin. Dan Med Bull. 2007;54:266–88.

29. Kageyama K, Tozawa F, Horiba N, Watanobe H, Suda T. Serotonin stimulates corticotropin-releasing factor gene expression in the hypothalamic paraventricular nucleus of conscious rats. Neurosci Lett. 1998;243:17–20.

30. Höglund K, Häggström J, Hanås S, Mervelle AC, Gouni V, Wiberg M. Inter-breed variation in serum serotonin (5-hydroxytryptamine) concentration in healthy dogs. J Vet Cardiol. 2018;20:244–53.

31. Palazzolo DL, Quadri SK. The effects of aging on circadian rhythm of serum cortisol in the dog. Exp Gerontol. 1987;22:379–87.

32. Giannetto C, Fazio F, Assenza A, Alberghina D, Panzera M, Piccione G. Parallelism of circadian rhythmicity of salivary and serum cortisol concentration in normal dogs. J Appl Biomed. 2014;12:229–33.

33. Thun R, Eggenberger E, Zerobin K. 24-hour profiles of plasma-cortisol and testosterone in the male dog—absence of circadian rhythmicity, seasonal influence and hormonal interrelationships. Reprod Domest Anim. 1990;25:68–77.

34. Castillo VA, Blatter MFC, Gomez NV, Sinatra V, Gallelli MF, Ghersevich MC. Diurnal ACTH and plasma cortisol variations in healthy dogs and in those with pituitary-dependent Cushing's syndrome before and after treatment with retinoic acid. Res Vet Sci. 2009;86:223–9.

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