Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Different Levels of Urinary Cortisol and Creatinine Presented at Different Time Points between Intact Male Dogs and Sterilized Dogs

Wenyuan Xue[1], Tien-Huan Hsu[1,2], Hsein-Chi Wang[1,2], Kuan-Sheng Chen[1,2] and Wei-Ming Lee[1,2*]

[1]Department of Veterinary Medicine; [2]Veterinary Medical Teaching Hospital, College of Veterinary Medicine, National Chung Hsing University, Taichung, Taiwan, R.O.C. 145, Xingda Rd., Taichung city, Taiwan, R.O.C.
*Corresponding author: wmlee@dragon.nchu.edu.tw

ARTICLE HISTORY (17-045)

Received: February 13, 2017
Revised: April 11, 2017
Accepted: May 11, 2017
Published online: May 18, 2017

Key words:
Cortisol
Creatinine
UCCR
Urine

ABSTRACT

The level of urine cortisol and creatinine is used for screening of hyperadrenocorticism, however, there was only few information concerning the effect of sex hormone on level of urinary cortisol and creatinine in dogs. The aim of this study was to investigate the level of urine cortisol and creatinine in intact male and neutered dogs and provide the optimal time of urine collection for screening of hyperadrenocorticism in dogs. The urine cortisol concentration in the intact group was significantly higher than the neutered one at 8:00 ($P<0.05$). In the intact group, urinary creatinine was decreasing from 8:00 to 16:00 of three consecutive days, though there was no statistical difference between each time. Urine creatinine was ascending in the neutered group from 8:00 to 16:00 with no significant difference at each time. In addition, the urinary creatinine concentration of neutered group was significantly higher than intact group at 16:00 ($P<0.05$). For the total mean urinary cortisol and creatinine ratios (UCCRs) of three consecutive days, intact group was significantly higher than the neutered group ($P<0.05$). There was no significant difference of UCCRs between neutered group and intact group at 8:00, while the UCCRs of intact group was significant higher than the neutered group at 12:00 and 16:00 ($P<0.05$). UCCR was significantly positive linear correlated to urine cortisol ($r=0.568$, $P<0.001$) but significantly negative linear correlated to creatinine ($r=-0.5009$, $P<0.001$). Thus, we can recommend that the optimal time of urine collection for UCCR was at 8:00 in the morning.

To Cite This Article: Xue W, Hsu TH, Wang HC, Chen KS and Lee WM, 2017. Different levels of urinary cortisol and creatinine presented at different time points between intact male dogs and sterilized dogs. Pak Vet J, 37(3): 350-354.

INTRODUCTION

Cortisol is the primary glucocorticoids released by zona fasciculate and zona reticularis of the adrenal cortex in canine, and the regulation of adrenal cortisol secretion is mainly regulated by the hypothalamus-pituitary-adrenocortical axis (HPA) (Chung *et al.*, 2011). However, recently studies have found that apart from the Adrenocorticotropic hormone (ACTH)-dependent mechanism, the cortisol is also modulated by the ACTH-independent mechanism. Actually, many other factors such as neuropeptides, adipokines, neurotransmitters, cytokine and intra-adrenal paracrine can influence the cortisol secretion. Meanwhile, septicemia that may cause the disorder of the cortisol which was not due to the ACTH influence (Bornstein *et al.*, 2008). Recently, sexual dimorphism of HPA has been described in different species under physiological conditions. Studies about the molecular bases of the sex-related differences found that genes involved in steroid hormone metabolism and their expression levels in the zona fasciculate/reticularis were significantly enriched in adult female than on males and companied by lower expression levels of genes regulating basal cell functions as cell fraction, oxidation/reduction processes (Trejter *et al.*, 2015a). In males, testosterone as the major androgen hormone principally produced by testicles is also similar as estrogen regulated by luteinizing hormone (LH) and follicle-stimulating hormone (FSH). While in the study of salivary cortisol concentration in healthy dogs found that cortisol values from intact dogs did not differ between males and females, whereas for cortisol in castrated males and spayed females significantly lower than intact dogs (Sandri *et al.*, 2015).

Sterilization known as spaying females and neutering males is commonly performed in the last few decades in

vet to decrease the risk of some sex hormone-related diseases such as mammary gland tumor (Spoerri *et al.*, 2015). Changes in hormonal regulation may occur after sterilization. In fact, the higher level of ACTH has been documented in ovariohysteectomized rats (Lin *et al.*, 2015). The changed hypothalamus hormones may (Frank *et al.*, 2003) or may not (Álvarez-Rodríguez *et al.*, 2016) affect the secretion of cortisol which is excreted by kidney (Rooney *et al.*, 2007). Therefore, urinary free cortisol can reflect the integrated cortisol production.

Creatinine is a metabolic product of the muscles in mammals and maintained by kidney which filters out most of the creatinine by glomerular filtration with little or no tubular reabsorption, and disposition of it in the urine (Cocker *et al.*, 2011). As the muscle mass is relatively constant from day to day, therefore, the creatinine production remains essentially unchanged on a daily basis, which is just depended on the muscle mass of the body. Besides, as creatinine is the relative stability within an individual that makes it an attractive approach for normalization of analyte concentrations for the effects of fluid balance. Urine cortisol and creatinine (UCCR) are used for screening or diagnosis of hyperadrenocorticism (Jensen *et al.*, 1997). However, the effect of sex hormone on level of urinary cortisol and creatinine in dogs is unclear, although cortisol values in the salivary of intact dogs did not differ between males and females, whereas for castrated males and spayed females the values were found to be significantly lower (Sandri *et al.*, 2015). Therefore, the aim of this study is to investigate the level of urine cortisol and creatinine in intact male and neutered dogs and provide the optimal time of urine collection for screen of hyperadrenocorticism in dogs.

MATERIALS AND METHODS

Animals: This study has been approved by Institutional Animal Care and Use Committee (IACUC No.105009) of National Chung Hsing University. Twelve healthy dogs which have six sterilized dogs and six intact male dogs were recruited from 12 private owners. The criteria for dogs to be enrolled into this study as following: (1) clinically healthy, free from pain; (2) without history of neurologic abnormalities; (3) no recent history of corticosteroid administration; and (4) no drug therapy for at least one month before sampling. Clinical data were obtained from the owners and the medical records in National Chung Hsing University Veterinary Teaching Hospital.

Experiment design: Twelve healthy dogs (n=12) housed in the acquainted environment, and were divided into 2 groups (n=6/group): (1) six neutered dogs; (2) six intact male dogs. All dogs are not suffering of behavioral disorders and acquainted to the environment, management, and housing.

Urine sample collection: The midstream voided urine samples were taken by each owner at home for three consecutive days. The dogs were taken outside and walked at 8:00, 12:00 and 16:00 every day. Each urine sample was collected and frozen at -70°C until analysis.

Urine cortisol concentration measurement: For each urine sample, homogenize and dispense 1 mL of the sample into each tube. Add 1 mL of dichloromethane to each tube. Shake for 20 to 30 seconds using a vortex-type mixer (G 560, Scientific Industries Inc., USA). Aspirating the aqueous phase when the two phases have separated. Collect 150 µL of the organic layer (dichloromethane) using a saturated tip and place in a pre-marked glass tube. Dry this solution in laminar flow hood. Dissolving each dry extract in 150 µL of serum free (Biomerieux, France) and leave to incubate for 30 minutes at room temperature. Shake immediately on a vortex-type mixer again before performing the assay. Adding 100 µL sample into the kit then initiate the assay immediately. All the assay steps were performed automatically by the mini VIDAS® (Biomerieux, France). Cortisol level was reported in ng/mL.

Urine creatinine concentration measurement: The urine samples were brought to room temperature then diluted 1 µL urine with 49 µL distilled water. Add 1000 µL 0.2 mol/L sodium hydroxide to sample, then mix to incubate for 5 minutes. Add 250 µL 20 mmol/L picric acid to read absorbance A1 after 60 seconds, read absorbance A2 after further 120 seconds by DiaSys Diagnostic system (GmBH, Germany).

The measurement of urinary cortisol/creatinine ratio: UCCR=UCo*31.2/UCr (UCo: urinary cortisol concentration; UCr: urinary creatinine concentration)

Statistical analysis: All data were expressed as mean±SEM. Differences between groups were tested for statistical significance by Student t-test. A P value<0.05 was considered as significant difference.

RESULTS

Totally, 102 urine samples were collected from the 12 healthy dogs. The urine cortisol concentration in the intact group was significantly higher than that in neutered dogs at 8:00 ($P<0.05$) (Fig. 1A). However, urine creatinine levels of neutered dogs were significantly higher than intact group at 16:00 ($P<0.05$) (Fig 1B). For the mean UCCR of three consecutive days, intact group was significantly higher than that in the neutered group ($P<0.05$) (Fig. 2A). No significant difference of UCCRs was noted between neutered group and intact group at 8:00, while the UCCRs of intact group were significant higher than that in the neutered group at 12:00 and 16:00 ($P<0.05$) (Fig. 2B). In addition, UCCR presented significantly positive correlation to urine cortisol ($r=0.568$, $P<0.001$) (Fig. 3A) and negative correlation to urine creatinine ($r=-0.5009$, $P<0.001$) (Fig. 3B). There was no correlation between ages and mean UCCR among these dogs (Fig. 4).

DISCUSSION

In this study, urinary cortisol concentration was significantly lower at 8:00 in neutered group than that in intact male group. It has been reported that high urinary cortisol has been reported in men compared to women (Ragnarsson *et al.*, 2015). It may indicate cortisol excreted

from urine is associated with sexual hormone. Apart from urine, cortisol concentration can be determined from saliva in dogs (Sandri *et al*., 2015). The salivary cortisol levels showed significantly lower in castrated males and spayed females dogs compared to that of intact dogs (Sandri *et al*., 2015). Therefore, lower urinary cortisol levels may indicate that the production of cortisol by adrenal gland is decreased in the neutered dogs.

It is well known the cortisol secretion by adrenal cortex is stimulated by ACTH and shows episodic secretion in dogs (Kemppainen *et al*., 1984). Cortisol concentrations are highest at 16:00 and maintained almost on a plateau until 20:00 (Castillo *et al*., 2009). The cortisol secretion pattern coincides with the secretion of ACTH by hypothalamus (Castillo *et al*., 2009). The number of secretory pluses in the dog was reported to be around six to twelve per 24-hour period (Kemppainen *et al*., 1984). Some unconjugated cortisol excreted in the urine in urine reflected the rhythmic variations of cortisol secretion (Beisel *et al*., 2016).

Various cortisone levels can be found in breeds of dogs (Höglund *et al*., 2016), and between sexes (Sherman *et al*., 2016). Actually, gonadal hormone can affect the cortisone secretion by adrenal gland, although there were only few reports about sex-related variation in adrenal hormone in dogs. Recently, in the studies of cattle, which found the proportion of free cortisol in circulation of bulls was greater than in steers (Ward *et al*., 1992) which may indicate lacking of gonadal hormone may cause negative feedback to the pituitary, hypothalamus or higher level than the HPA axis (Ward *et al*., 1992) and be less the effect on adrenal receptor (Trejter *et al*., 2015[b]) that may have some effects on the production of adrenal cortisol. In addition, the lower concentration of urine cortisol in neutered dogs may be due to the decrease of growth hormone after sterilization. It has been reported that sterilization cause markedly decreased growth hormone (GH) production and secretion. Meanwhile, some studies have shown that physiological amounts of glucocorticoids are required for normal GH (Giustina, 1994).

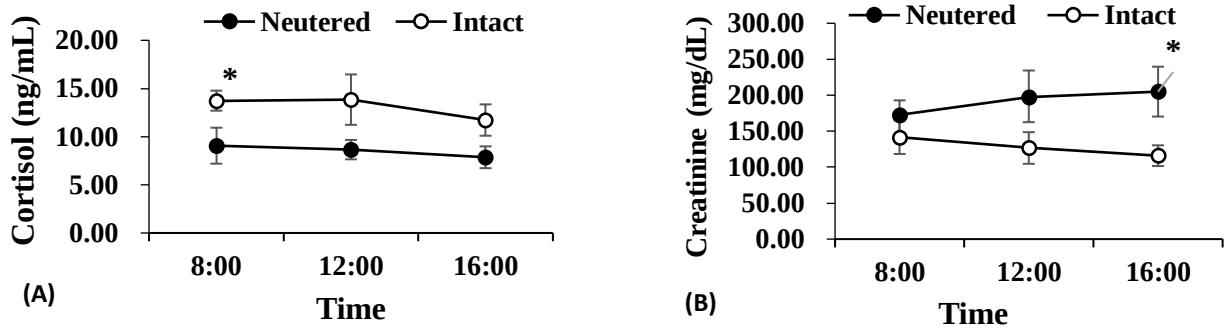

Fig. 1: (A) Mean cortisol±SEM of three days in neutered dogs and intact male dogs; (B) Mean creatinine±SEM of three days in neutered dogs and intact male dogs *P<0.05.

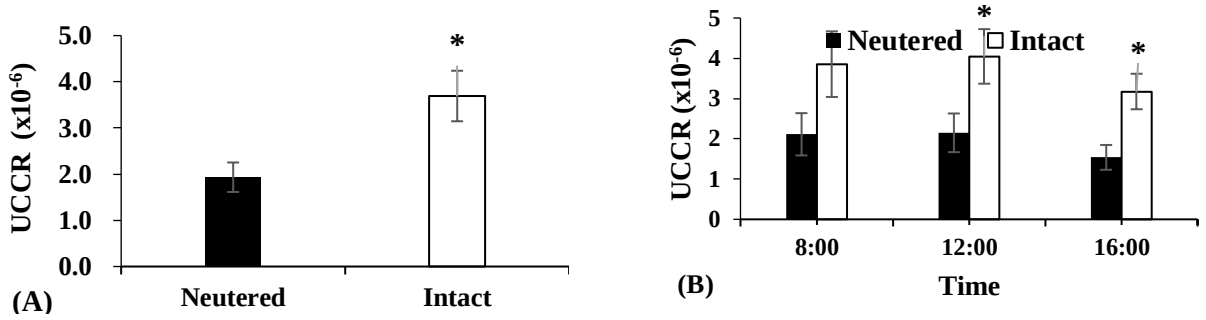

Fig. 2: (A) Bar graph representation of total mean UCCR±SEM derived from all urine samples collected from neutered dogs and intact male dogs; (B) Mean UCCR±SEM of three days in neutered dogs and intact male dogs *P<0.05.

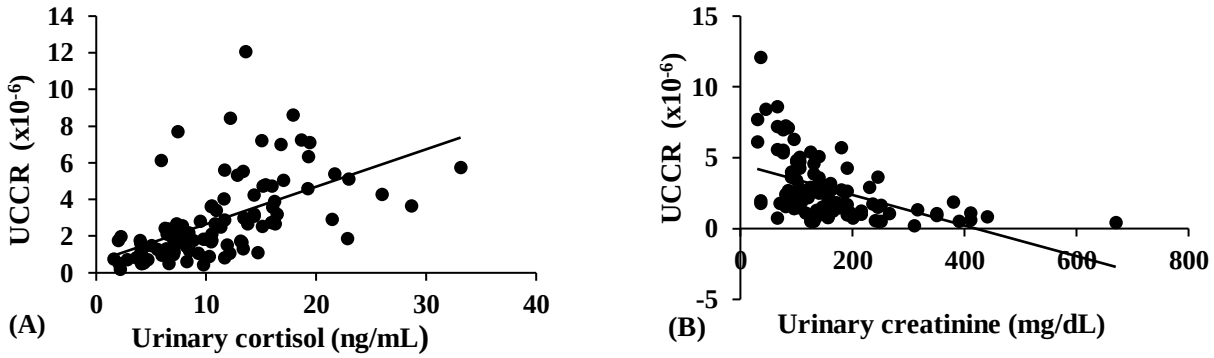

Fig. 3: (A) Correlation between UCCR and UCo (urinary cortisol) in multiple voided samples (n=108) from twelve healthy dogs. The equation of linear regression is UCCR= 0.6147 + 0.2041(UCo). (B) Correlation between UCCR and UCr (urinary creatinine) in all urine samples collected from twelve healthy dogs. The equation of linear regression is UCCR= -0.0108 + 4.538(UCr).

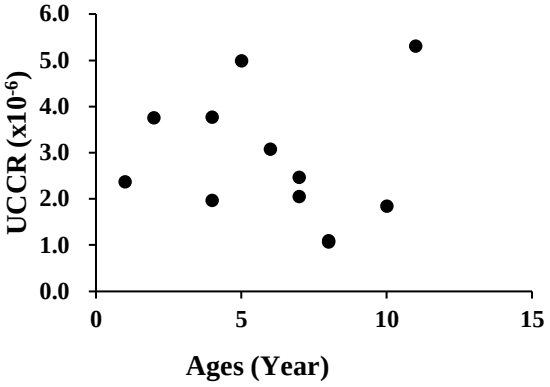

Fig. 4: Lack of correlation between UCCR and ages in twelve healthy dogs.

In this study, urinary creatinine concentrations of neutered dogs were significantly higher than that in intact male dogs at 16:00. Creatinine excreted in urine has been accepted as an indicator of catabolism of muscle mass which may be associated with GH. Actually, GH as one of the most potent anabolic agents may diminish muscle catabolism and was effective in decreasing urinary creatinine excretion (Akçay *et al.*, 2001).

It is well known, urinary cortisol levels can reflect the elevated levels of plasma cortisol (Rooney *et al.*, 2007) and combine with urinary creatinine levels being used for screening hyperadrenocorticism in dogs (Feldman and Mack, 1992; Jensen *et al.*, 1997). In the results of this study, UCCR was positively correlated to the levels of urinary cortisol but negatively correlated to the level of urinary creatinine. The UCCR was significantly higher in intact male dogs at 12:00 and 16:00 than those of neutered ones. However, there was no significant difference in UCCR at 8:00 between intact male and neutered dogs in this study. It may indicate that the optimal time for urine collection in dogs was at 8:00 which was consistent with previous study (Galac *et al.*, 2009).

In this study, there was no significant difference in age between these two groups which was similar to the previous study (Galeandro *et al.*, 2014). Some reports indicated an age-related increase in circulating cortisol (Rothuizen *et al.*, 1993; Strasser *et al.*, 1993; Goy-Thollot *et al.*, 2007) and others did not (Palazzolo *et al.*, 1987; Reimers *et al.*, 1990; Mongillo *et al.*, 2014). Thus, age-related influence on cortisol production is still unclear.

Conclusions: Urinary cortisol concentration was higher in the intact male dogs than that in the neutered male ones at 8:00, while the neutered male dogs had significantly higher levels of urinary creatinine at 16:00. Besides, there was no age-related influence on cortisol production. There was no significant difference of UCCR at 8:00 in this study. Thus, urine samples for UCCR detection at 8:00 in the morning is preferred.

Authors contribution: WX and WL conceived and designed the study. TH, KC, HW and WL executed the experiment and KC and HW analyzed the sera and tissue samples. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version IJ analyzed the data.

REFERENCES

Akçay MN, Akçay G, Solak S, *et al*., 2001. The effect of growth hormone on 24-h urinary creatinine levels in burned patients. Burns 27:42-5.

Álvarez-Rodríguez J, Albertí P, Ripoll G, *et al*., 2016. Castration at 10 months of age on growth physiology and behavior of male feral beef cattle. Anim Sci J doi: 10.1111/asj.12728. [Epub ahead of print]

Beisel WR, Cos JJ, Horton R, *et al.*, 2016. Physiology of urinary cortisol excretion 24:887-93.

Bornstein SR, Engeland WC, Ehrhart-Bornstein M, *et al.*, 2008. Dissociation of ACTH and glucocorticoids. Trends Endocrinol Metab 19:175-80.

Castillo VA, Cabrera Blatter MF, Gómez NV, *et al.*, 2009. 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 86:223-9.

Chung S, Son GH and Kim K, 2011. Adrenal peripheral oscillator in generating the circadian glucocorticoid rhythm. Ann N Y Acad Sci 1220:71-81.

Cocker J, Mason H, Warren N, *et al.*, 2011. Creatinine adjustment of biological monitoring results. Occup Med (Lond) 61:349-53.

Feldman EC and Mack RE, 1992. Urine cortisol: creatinine ratio as a screening test for hyperadrenocorticism in dogs. J Am Vet Med Assoc 200:1637-41.

Frank L, Rohrbach B, Bailey E, *et al.*, 2003. Steroid hormone concentration profiles in healthy intact and neutered dogs before and after cosyntropin administration. Domest Anim Endocrinol 24:43-57.

Galac S, Buijtels JJ and Kooistra HS, 2009. Urinary corticoid: creatinine ratios in dogs with pituitary-dependent hypercortisolism during trilostane treatment. J Vet Intern Med 23:1214-9.

Galeandro L, Sieber Ruckstuhl NS, Riond B, *et al.*, 2014. Urinary corticoid concentrations measured by 5 different immunoassays and gas chromatography mass spectrometry in healthy dogs and dogs with hypercortisolism at home and in the hospital. J Vet Intern Med 28:1433-41.

Giustina A, 1994. Reciprocal relationship between the level of circulating cortisol and growth hormone secretion in response to growth hormone-releasing hormone in man: studies in patients with adrenal insufficiency. J Clin Endocrinol Metab 79:1266-72.

Goy-Thollot I, Decosne-Junot C and Bonnet J, 2007. Influence of aging on adrenal responsiveness in a population of eleven healthy beagles. Res Vet Sci 82:195-201.

Höglund K, Lequarré AS, Ljungvall I, *et al.*, 2016. Effect of breed on plasma endothelin-1 concentration, plasma renin activity, and serum cortisol concentration in healthy dogs. J Vet Intern Med 30:566-73.

Jensen AL, Iversen L, Koch J, *et al.*, 1997. Evaluation of the urinary cortisol: creatinine ratio in the diagnosis of hyperadrenocorticism in dogs. J Small Anim Pract 38:99-102.

Kemppainen RJ and Sartin JL, 1984. Evidence for episodic but not circadian activity in plasma concentrations of adrenocorticotrophin, cortisol and thyroxine in dogs. J Endocrinol 103:219-26.

Lin JL, Shiu LS, Wang HC, *et al.*, 2015. Response of GH, ACTH, TSH AND IGF-1 to gonadotropin releasing hormone stimulation in ovariohysterectomized rats. Thai J Vet Med 45:331-339.

Mongillo P, Prana E, Gabai G, *et al.*, 2014. Effect of age and sex on plasma cortisol and dehydroepiandrosterone concentrations in the dog (*Canis familiaris*). Res Vet Sci 96:33-8.

Palazzolo DL and Quadri SK, 1987. Plasma thyroxine and cortisol under basal conditions and during cold stress in the aging dog. Proc Soc Exp Biol Med 185:305-11.

Ragnarsson O, Trimpou P, Oleröd G, *et al.*, 2015. The association between urinary cortisol excretion and cardiovascular risk factors, bone status and quality of life in the population. Steroids 101:71-7.

Reimers TJ, Lawler DF, Sutaria PM *et al.*, 1990. Effects of age, sex, and body size on serum concentrations of thyroid and adrenocortical hormones in dogs. Am J Vet Res 51:454-457.

Rooney NJ, Gaines SA and Bradshaw JWS, 2007. Behavioral and glucocorticoid responses of dogs (Canis familiaris) to kenneling: Investigating mitigation of stress by prior habituation. Physiol Behav 92:847-54.

Rothuizen J, Reul JM, van Sluijs FJ, *et al.*, 1993. Increased neuroendocrine reactivity and decreased brain mineralocorticoid receptor-binding capacity in aged dogs. Endocrinology 132:161-8.

Sandri M, Colussi A, Perrotta M, *et al.*, 2015. Salivary cortisol concentration in healthy dogs is affected by size, sex, and housing context. J Vet Behav: Clin Appl Res 10:302-6.

Sherman GD, Rice LK, Jin ES, *et al.*, 2016. Sex differences in cortisol's regulation of affiliative behavior. Horm Behav, pii: S0018-506X (16)30026-5. doi: 10.1016/j.yhbeh.2016.12.005. [Epub ahead of print]

Spoerri M, Guscetti F, Hartnack S, *et al.*, 2015. Endocrine control of canine mammary neoplasms: serum reproductive hormone levels and tissue expression of steroid hormone, prolactin and growth hormone receptors. BMC Vet Res 11:235.

Strasser A, Niedermüller H, Hofecker G, *et al.*, 1993. The effect of aging on laboratory values in dogs. Zentralbl Veterinarmed A 40:720-30.

Trejter M, Hochol A, Tyczewska M, *et al.*, 2015a. Sex-related gene expression profiles in the adrenal cortex in the mature rat: microarray analysis with emphasis on genes involved in steroidogenesis. Int J Mol Med 35:702-14.

Trejter M, Jopek K, Celichowski P, *et al.*, 2015b. Expression of estrogen, estrogen related and androgen receptors in adrenal cortex of intact adult male and female rats. Folia Histochem Cytobiol 53:133-44.

Ward JR, Henricks DM, Jenkins TC, *et al.*, 1992. Serum hormone and metabolite concentrations in fasted young bulls and steers. Domest Anim Endocrinol 9:97-103.