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Validation of a noninvasive diagnostic tool to verify neuter status in dogs: The urinary FSH to creatinine ratio

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ABSTRACT

Determining the presence of functional gonadal tissue in dogs can be challenging, especially in bitches during anestrus or not known to have been ovariectomized, or in male dogs with nonscrotal testes. Furthermore, in male dogs treated with deslorelin, a slow-release GnRH agonist implant for reversible chemical castration, the verification of complete downregulation of the hypothalamic-pituitary-gonadal (HPG) axis can be difficult, especially if pretreatment parameters such as the size of the testes or prostate gland are not available. The aims of this study were to validate an immunoradiometric assay for measurement of FSH in canine urine, to determine if the urinary FSH to creatinine ratio can be used to verify the neuter status in bitches and male dogs, as an alternative to the plasma FSH concentration, and to determine if downregulation of the HPG axis is achieved in male dogs during deslorelin treatment. Recovery of added canine FSH and serial dilutions of urine reported that the immunoradiometric assay measures urinary FSH concentration accurately and with high precision. Plasma FSH concentrations (the mean of two samples, taken 40 minutes apart) and the urinary FSH to creatinine ratio were determined before gonadectomy and 140 days (median, range 121–225 days) and 206 days (median, range 158–294 days) after gonadectomy of 13 bitches and five male dogs, respectively, and in 13 male dogs before and 132 days (median, range 117–174 days) after administration of a deslorelin implant. In both bitches and male dogs, the plasma FSH concentration and the urinary FSH to creatinine ratio were significantly higher after gonadectomy, with no overlapping of their ranges. Receiver operating characteristic analysis of the urinary FSH to creatinine ratio revealed a cut-off value of 2.9 in bitches and 6.5 in males to verify the presence or absence of functional gonadal tissue. In male dogs treated with deslorelin, the plasma FSH concentrations and urinary FSH to creatinine ratios were significantly lower after administration of the implant, but their ranges overlapped. We conclude that the urinary FSH to creatinine ratio can be used to verify the neuter status of bitches and male dogs. However, it cannot be used for the assessment of complete downregulation of the HPG axis after administration of a deslorelin implant. The urinary FSH to creatinine ratio is preferable over the plasma FSH concentration because it involves only one sample that can be collected relatively easy and noninvasively.

1. Introduction

The bitch has a nonseasonal, monestrous cycle characterized by a follicular phase with spontaneous ovulations, followed by a luteal phase of about 2 months, almost

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irrespective of whether the bitch is pregnant, and a nonseasonal anestrus of 2 to 10 months [1]. As in other species, the endocrine control of reproduction is exerted by the hypothalamic-pituitary-gonadal (HPG) axis. Gonadotropic releasing hormone (GnRH) is secreted by the hypothalamus and stimulates the gonadotropes of the pituitary gland to secrete LH and FSH. These stimulate the gonads to produce sex steroids, which exert negative and/or positive feedback on the hypothalamus through interaction with kisspeptin neurons. Kisspeptin directly stimulates GnRH neurons to secrete GnRH [2,3]. The secretion of GnRH is pulsatile and thus secretion of LH and FSH is also pulsatile, as is that of all hormones secreted by the adenohypophysis [4,5].

When it is unknown whether an ovariectomy has been performed, the presence of functional gonadal tissue in bitches can easily be verified by vaginoscopy and/or vaginal cytology during proestrus and estrus, revealing changes induced by high-circulating levels of estradiol, and by measuring plasma progesterone concentration during the luteal phase. However, it may be difficult to distinguish ovariectomized from intact bitches during anestrus [6]. Abdominal ultrasound may be used to determine if ovaries are present or not. However, the sensitivity and specificity of this technique depends on the level of experience of the sonographer and the specifications of the equipment used, for example, the transducer [7]. During the follicular phase and luteal phase, it is more likely that the ovaries can be identified by using abdominal ultrasound because of the presence of follicles and corpora lutea respectively. In contrast, during anestrus, the ovaries are smaller and have a more homogenous appearance (absence of large follicles and corpora lutea) and are therefore more challenging to identify, especially in large dogs or in dogs with a large amount of abdominal fat tissue [7,8]. In addition, Buijtels et al.[9] described two populations of dogs: bitches with remnant ovarian tissue and dogs with a disorder of sexual development [10]. In both studies, abdominal ultrasound was used to verify the presence and localization of gonadal tissue in addition to a provocative GnRH stimulation test, and thereafter laparotomy and histology of the surgically removed tissue was performed. In both studies, it appeared that the results of the abdominal ultrasound were not always conclusive. Therefore, the use of abdominal ultrasound alone cannot be used to either confirm or rule out the presence of functional ovarian tissue.

In both males and females, mean basal plasma LH and FSH concentrations increase after gonadectomy, due to loss of negative feedback of gonadal hormones [11–13]. Basal plasma FSH concentration is higher in ovariectomized bitches than in intact anestrous bitches, and the ranges do not overlap [12,13]. In contrast, the ranges of basal plasma LH and estradiol concentration in ovariectomized bitches and intact anestrous bitches do overlap [12,13]. On the basis of these findings, measurement of plasma FSH concentration appears to be the most reliable means of verifying the presence of ovarian tissue during anestrus. However, a single measurement of plasma FSH concentration may not be reliable for this purpose because gonadotropin secretion is pulsatile. Kooistra et al.[4] (1999) have reported that there are one to two LH and FSH pulses per 6 hours during the luteal phase and anestrus. Hence, multiple blood samples are required to circumvent the effect of pulsatile secretion on the interpretation of gonadotropin concentrations.

Another means of verifying the neuter status of dogs is the measurement of plasma anti-mullerian-hormone (AMH) concentration. The ovaries are considered to be the sole source of AMH, and therefore, it can be expected that AMH will be a specific indicator of the presence of functional gonadal tissue. Plasma AMH concentration has been shown to be higher in intact dogs than in ovariectomized dogs but with some overlapping of the ranges [14]. Because of the overlapping and the large individual differences in plasma gonadotropin, estradiol, and AMH concentrations, the reliability of single measurement of the plasma concentration of one of these hormones during anestrus may not be sufficient to verify neuter status.

Provocative tests with exogenous GnRH can also be used to detect the presence of functional ovarian tissue. After intravenous injection of a GnRH analog, a significant rise in plasma estradiol concentration is observed in anestrous bitches but not in ovariectomized bitches [6]. However, the GnRH stimulation test has the disadvantages of being invasive, more than one blood sample is needed, and somewhat time consuming (maximum plasma estradiol concentration after GnRH injection is only reached after 60–120 minutes) [6,13].

Measurement of the FSH concentration in urine may be a useful alternative to determine the presence of functional ovarian tissue during anestrus. It reflects the plasma FSH concentration over a period of several hours and therefore is expected to compensate for pulsatile FSH secretion. To correct for changes in concentration of the urine, which influences the urinary FSH concentration, the urinary FSH to creatinine ratio should be used, in analogy to the previously validated corticoid to creatinine ratio [15]. Compared with other methods to verify the presence of functional gonadal tissue, for example a GnRH stimulation test, the collection of urine for measurement of the FSH concentration is easy and noninvasive.

Basal plasma testosterone concentration is much higher in intact male dogs than in gonadectomized males, and the ranges do not overlap [13]. Consequently, a single measurement of plasma testosterone concentration is a reliable means of verifying the presence of functional testicular tissue as, for example, when undescended testes are suspected and the dogs' history is unknown. It is more difficult to determine the functionality of the testicles during temporary chemical castration by the use of a slow-release GnRH agonist (deslorelin) implant. The GnRH receptor on the gonadotropes becomes desensitized after long-term stimulation [16]. The duration of downregulation of the HPG axis is dependent on the dose of deslorelin, albeit with large interindividual variation. The time to complete recovery of the HPG axis has been reported to vary between 360 and 680 days after implantation of 6-mg deslorelin [17]. Downregulation of the GnRH receptors on the gonadotropes results in a decrease in the plasma LH concentration [18]; effects on plasma FSH concentration have not been reported. To evaluate the function of the testicular tissue in a male dog treated with a