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Original article

The evaluation of blood concentrations of testosterone, 17β-oestradiol and anti-Müllerian hormone in dogs with cryptorchidism and testicular tumours

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Abstract

Cryptorchidism and testicular tumours are very common disorders in dogs genitalia. The aim of the present study was o obtain an overview of serum 17β-oestradiol, anti-Müllerian hormone (AMH) and testosterone levels in intact dogs compared to dogs with different testicular tumours and dogs with cryptorchidism. Testosterone, AMH and 17β-oestradiol concentrations were measured in peripheral and local spermatic venous blood in dogs with unilateral cryptorchidism (n=8), dogs with neoplastic testicular diseases (n=8) and in a control group of mature intact dogs (n=8). Results confirmed significantly higher concentrations of testosterone in local venous blood (control group: right testicle (RT) 46.23 ± 40.88 ng/ml and left testicle (LT) 50.76 ± 43.76 ng/ml; cryptorchid group: RT 23.91 ± 22.79 ng/ml and LT 10.52 ± 7.37 ng/ml; tumour group: RT 37.26 ± 25.26 ng/ml and LT 44.86 ± 19.03 ng/ml) ($p<0.05$) compared to their concentrations in peripheral blood (PB) in a control (4.92 ± 3.3 ng/ml) and in a cryptorchid group (0.89 ± 0.78 ng/ml), but not in the tumour group (11.37 ± 10.86 ng/ml). However, we have found increased level of testosterone in PB in the tumour group compared to its PB concentrations in the control or the cryptorchid group. Concentrations of AMH in PB observed in the cryptorchid group was 54.98 ± 30.07 µg/ml and in the control group was 6.49 ± 3.24 µg/ml ($p<0.05$). The same was observed in the case of local blood concentrations, which were significantly higher in the cryptorchid group (RT 51.92 ± 30.59 µg/ml; LT 46.33 ± 34.86 µg/ml) ($p<0.05$). We also observed high oestradiol concentrations in the cryptorchid group in both peripheral and local blood (PB: 30.86 ± 20.28 pg/ml; RT: 55.71 ± 34.7 pg/ml; LT: 78.99 ± 47.72 pg/ml), and even higher in the tumour group (PB: 52.46 ± 34.02 pg/ml; RT: 188.16 ± 132.67 pg/ml; LT: 297.14 ± 245.56 pg/ml). AMH has been shown to be a specific biomarker of gonadal tumours originated in Sertoli cells. It is also useful marker for confirmation of the existence of a functional cryptorchid testis. According to us, the scientific work dealing with a disorder of testicular descent in dogs, regarding the evaluation of sex hormones levels and the formation of the testes using modern diagnostic methods, significantly contribute to the clarification of some processes, leading to pathophysiological disturbances during this process.

Key words: dogs, testosterone, 17β-oestradiol, anti-Müllerian hormone, stimulation test

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Introduction

Epidemiological studies point to the fact that reproductive disorders in males have had an increasing trend in the last 50 years. The most common disorders of the male reproductive system include: cryptorchidism, hypospadia, poor quality of semen and testicular tumours (especially seminomas) and others (Skakkebaek et al. 2001).

Hormonal regulation plays a crucial role in the descent of the testicles (Hutson et al. 1997, Nef and Parada 2000, Kaleva and Toppari 2003). The plausible causes of cryptorchidism include insufficient disrupted endocrine regulation by androgens, AMH and insulin-like peptide 3 (INSL3) (Amann and Veeramachaneni 2007, Matuszczak et al. 2012). Cryptorchidism predisposes the animal to testicular neoplasia, therefore it is diagnostically essential that cryptorchidism is differentiated from other conditions in which testes are not present in the scrotum, including anorchidism or animals that have been castrated (Johnston et al. 2001). Strategies for diagnosis of cryptorchidism include palpation, ultrasound, or measurement of serum hormone levels. One of the endocrinological approaches to evaluation of the existence and functionality of the testis is the measurement of the plasma testosterone concentrations before and after GnRH stimulation. In the last decade, new possibilities for diagnosing cryptorchidism are enabled by analysis of AMH (Holst 2017).

Testicular tumours are the most common tumours of the canine male genitalia and are the second most common anatomic site for the tumour development in intact male dogs (Nødtvedt et al. 2011). Testicular tumours represent more than 90% of all canine male genital tumours and dogs have the highest incidence of all animal species (Huggins and Mouder 1945). Testicular tumours incidence, including the interstitial cells tumours, seminomas and Sertoli cells tumours is much higher in older dogs. Previously published hormonal analyses in dogs with testicular tumours have usually referred to single case reports (Metzger et al. 1993) or small series (Röcken et al. 1995, Quartuccio et al. 2012). They were generally based on selected subjects (Suess et al. 1992, Metzger et al. 1993, Röcken et al. 1995, Quartuccio et al. 2012), in which a clinically manifested feminization and/or bone marrow hypoplasia prompted the analysis of the sex hormones. Serum oestradiol concentration is used to diagnose hyperestrogenism in dogs suspected of having testicular tumours (Feldman et al. 2004). Measuring the concentrations of 17β-oestradiol and testosterone in dogs with the testicular tumours showed that the dogs with Sertoli cells tumours (SCT) had higher oestradiol levels and lower testosterone values compared to other types of testicular tumours (Mischke et al. 2002). The authors report that in healthy dogs variability in oestradiol levels was found, both between measurements and between dogs, and the concentrations may exceed the reference values. Therefore, although hyperestrogenism can cause many clinical problems, concentrations of oestradiol higher than the reference range may not be associated with clinical signs of hyperestrogenism (Mischke et al. 2002). With regard to that fact, SCT diagnosis requires measurement of 17β-oestradiol, along with other tests.

Anti-Müllerian hormone (AMH) is a specific and important regulator of gonadal function. It is produced exclusively by granulosa cells of small growing follicles in the ovaries and Sertoli cells in the testes in male dogs (Banco et al. 2012, Themmen et al. 2016). In mammals, anti-Müllerian hormone is the earliest specific protein expressed by Sertoli cells, also called Müllerian inhibiting substance (MIS), a glycoprotein that belongs to the transforming growth factors (TGF) of β-family. Sertoli cells produce high concentrations of AMH from the time of testicular differentiation up to puberty. Its main effect is the regression of Müllerian ducts at the initiation of male sex differentiation (Josso et al. 2001). After puberty, testosterone inhibits AMH production through the androgen receptor that is expressed by mature Sertoli cells (Josso et al. 2013). In dogs, AMH was expressed in Sertoli cells from foetuses and pups up to day 45 (Banco et al. 2012). Anti-Müllerian hormone in neutered males would indicate the possible presence of functionally normal or tumorous Sertoli cells (Themmen et al. 2016). In female dogs, AMH has been described to distinguish ovariohysterectomized from intact bitches (Place et al. 2011). AMH has been used to differentiate cryptorchidism from anorchidism in humans (Lee 1997). In addition, AMH level has been reported to be higher in cryptorchid stallions than in intact and castrated ones (Claes 2013).

The main aim of our study was to obtain an overview of serum 17β-oestradiol, AMH and testosterone levels in intact dogs compared to dogs with different testicular tumours and dogs with cryptorchidism. Hormonal analysis was performed on all male dogs, regardless of the clinical appearance.

Materials and Methods

The study was done on 24 dogs of different body weight and age which were classified into three groups: control group (intact healthy dogs, n=8), dogs with testicular tumors (n=8) and dogs with crypto-

rchidism (unilateral, subcutaneous, n=8). The dogs were presented to Small animal clinic for elective castration and castration for treatment of cryptorchidism or testicular tumour. All the dogs were privately owned, and the owners consent was obtained before the collection of samples. The study was conducted according to the regulations of the local Institutional Animal Care and Use Committee.

Stimulation test: The testosterone concentration in the blood serum of dogs was observed before the stimulation and 60 minutes after stimulation with exogenous synthetic GnRH, which was administered intravenously via the cephalic vein. Gonadoreline was used for the stimulation (Fertagyl, Intervet/ Schering-Plough Animal Health, Boxmeer, the Netherlands) in a dose of 10 µg/kg B.W.

Blood samples from *v. cephalica antebrachii* were placed to the test tubes with agglutinative gel using sterile single-use needles (21G x 38 mm, 0.8 x 38 mm). The blood was then centrifuged at 3500 rpm for 10 min (Eppendorf centrifuge 5702), where the blood serum was separated and stored at -24ºC until the analysis. Local venous blood from *plexus pampiniformis* from each testicle was collected and processed the same way.

Determination of testosterone: testosterone concentration was quantitatively determined in a specialized RIA laboratory using a RIA Testosterone commercial kit (Immunotech, Beckman Coulter Ltd., Prague, Czech Republic). Assay sensitivity indicated by the manufacturer was 0.02 ng/ml (analytical sensitivity), 0.08 ng/ml (functional sensitivity) and intra-assay and inter-assay coefficients of variation were less or equal to 5.6% and 15%, respectively.

Determination of 17β-oestradiol: 17β-oestradiol concentration was quantitatively determined in a specialized RIA laboratory using a Chemiluminescent Microparticle Immunoassay (ARCHITECT Estradiol, Abbott Laboratories, Abbott Park, Illinois, USA). Assay sensitivity indicated by the manufacturer was≤10 pg/ml (analytical sensitivity), ≤25 pg/ml (functional sensitivity) and coefficient of variation were less or equal to 20%.

Determination of AMH: In order to establish AMH concentration, we used immunochemical methods of electrochemiluminescence analysis (ECLIA). The evaluation of samples was performed using a Cobas E601 analyzer (Roche Diagnostics, Mannheim, Germany) in specialized laboratory. The Elecsys AMH assay is a sandwich assay based on electrochemiluminescence technology. The total duration of the assay is 18 minutes, and the sample volume is 50 µl. The assay is calibrated against the Beckman Coulter AMH Gen II ELISA (unmodified version without predilution) assay. The limit of quantitation (functional sensitivity) is 0.03 ng/ml. The coefficients of variation as determined for the control samples during the study measurements were ≤3.3% for the intermediate precision for AMH.

Statistical analysis: The quantitative data were presented as mean ± S.E.M. (Standard Error of Mean). The quantitative analysis was used for all analyzed parameters in each group. Multiple comparison procedures were performed by the One-Way Analysis of Variance (ANOVA) Student-Newman-Keuls method, using SigmaStat software (Jandel Corp. USA). P values equal to or <0.05 were considered significant.

Results

Individual measurements of testosterone, anti-Müllerian hormone and oestradiol concentrations in intact (control) dogs, dogs with cryptorchidism and dogs with tumour are shown in Figs. 1, 2 and 3, and the data are presented in Table 1.

Testosterone concentrations in the control groups measured in peripheral blood (PB: 4.92 ± 3.3 ng/ml) were found to be significantly lower than in local blood obtained from both, right testicle (RT: 46.23 ± 40.88 ng/ml) and left testicle (LT: 50.76 ± 43.76 ng/ml), ($p<0.05$). This difference was also observed in cryptorchid group (PB: 0.89 ± 0.78 ng/ml vs. RT: 23.91 ± 22.79 ng/ml; LT: 10.52 ± 7.37 ng/ml), although it was not as noticeable as in the control group. A significant difference was also found between the control group (4.92 ± 3.3 ng/ml) and the group with cryptorchidism measured in peripheral blood, being lower in cryptorchidic dogs (0.89 ± 0.78 ng/ml), ($p<0.05$). On the other hand, in dogs with tumours there was a significant increase in testosterone concentrations in peripheral blood (11.37 ± 10.86 ng/ml) compared to the control group or the cryptorchid group ($p<0.05$). Testosterone concentrations in local blood (in both, right and left testicles) in the control group (RT: 46.23 ± 40.88 ng/ml; LT: 50.76 ± 43.76 ng/ml) was found to be slightly higher than in cryptorchid (RT: 23.91 ± 22.79 ng/ml; LT: 10.52 ± 7.37 ng/ml) or the tumour group (RT: 37.26 ± 25.26 ng/ml; LT: 44.86 ± 19.03 ng/ml) (Fig. 1, Table 1).

Although the testosterone levels in the control and the cryptorchid group between peripheral blood and local blood were considerably different, concentrations of anti-Mullerian hormone in peripheral blood and local blood were practically the same in the control group (PB: 6.49 ± 3.24 µg/ml vs. RT: 6.25 ± 3.06 µg/ml; LT: 6.82 ± 3.09 µg/ml), in the cryptorchid group (PB: 54.98 ± 30.07 µg/ml vs. RT: 51.92 ± 30.59 µg/ml; LT: 46.33 ± 34.86 µg/ml) and in the

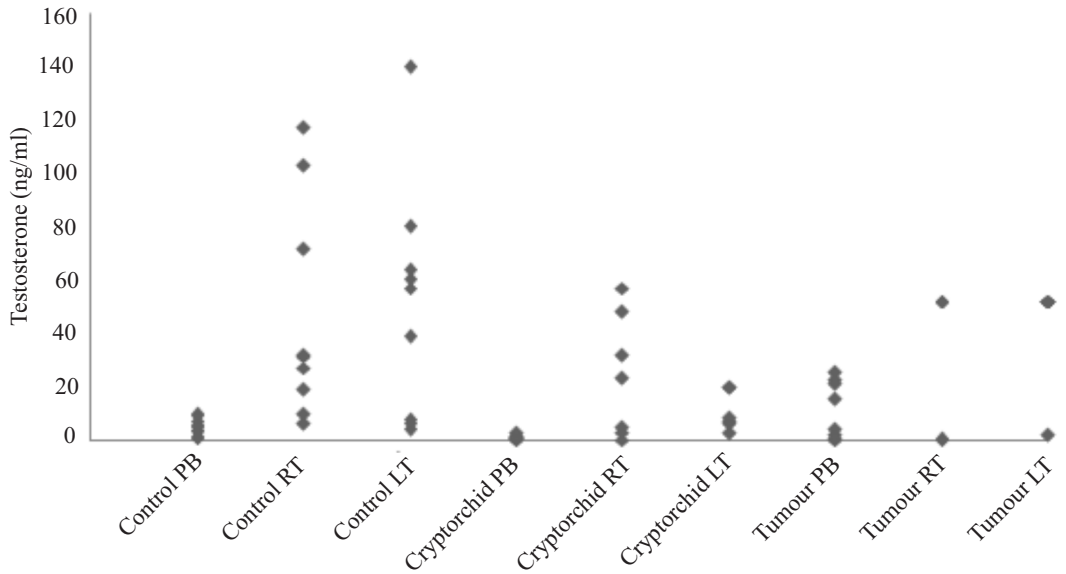

Fig. 1. Dot plot of testosterone concentrations in healthy dogs (Control), cryptorchid dogs (Cryptorchid) and dogs with tumour (Tumour). Obtained from peripheral blood (PB), local blood from right testicle (RT) and local blood from left testicle (LT). Number of measurements (n=8: Control PB, RT, LT, Cryptorchid PB, Tumour PB; n=7: Cryptorchid RT, Tumour RT, LT; n=6: Cryptorchid LT).Statistical significance ($p<0.05$): Control PB vs. Cryptorchid PB; Control PB vs. Tumour PB; Cryptorchid PB vs. Tumour PB; Control PB vs. Control RT; Control PB vs. Control LT; Cryptorchid PB vs. Cryptorchid RT; Cryptorchid PB vs. Cryptorchid LT; Control RT and Control LT vs. Tumour RT and Tumour LT.

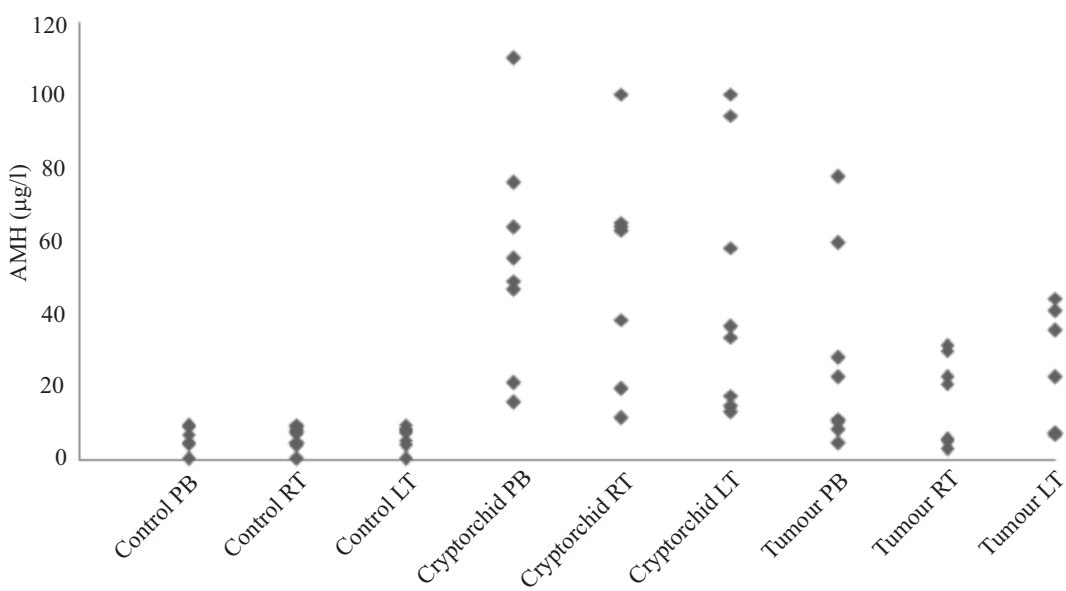

Fig. 2. Dot plot of anti-Mullerian hormone concentrations in healthy dogs (Control), cryptorchid dogs (Cryptorchid) and dogs with tumour (Tumour). Obtained from peripheral blood (PB), local blood from right testicle (RT) and local blood from left testicle (LT). Number of measurements (n=8: Control PB, RT, LT, Cryptorchid PB, LT, Tumour PB; n=7: Cryptorchid RT, Tumour RT, LT). Statistical significance ($p<0.05$): Control PB vs. Cryptorchid PB; Control RT and Control LT vs. Cryptorchid RT and Cryptorchid LT.

tumour group (PB: 28.13 ± 26.7 µg/ml vs. RT: 17.18 ± 12.5 µg/ml; LT: 23.9 ± 16.63 µg/ml). A significant increase in anti-Mullerian hormone concentrations in peripheral blood were observed in the cryptorchid group (54.98 ± 30.07 µg/ml) compared to the control group (6.49 ± 3.24 µg/ml), ($p<0.05$). Moreover, its concentrations in peripheral blood in the tumour group (28.13 ± 26.7 µg/ml) were higher than in the control group, but the difference was not significant. The same was observed in the case of local blood concentrations, being significantly higher in the cryptorchid group (RT: 51.92 ± 30.59 µg/ml;

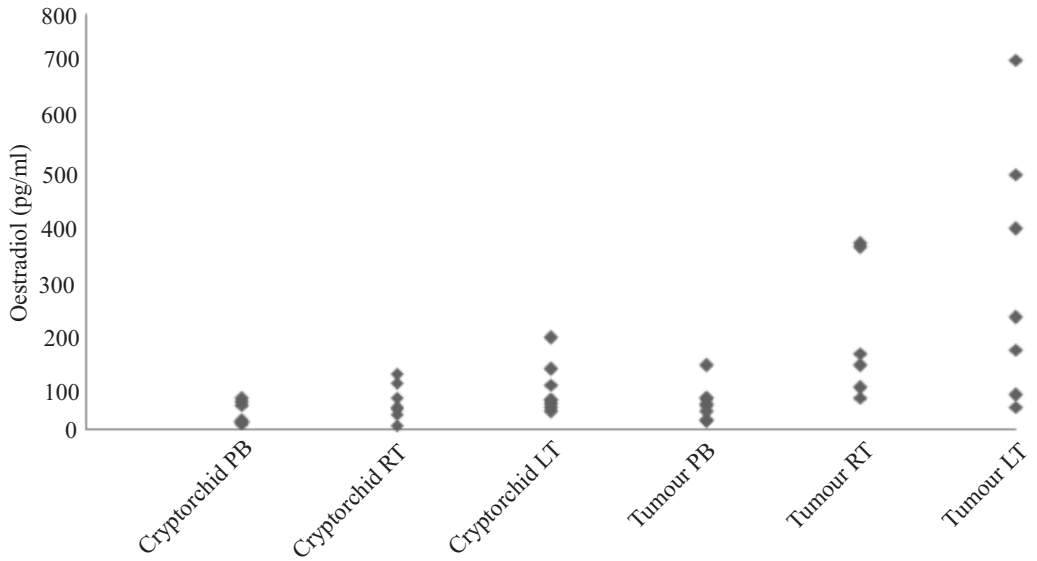

Fig. 3. Dot plot of Oestradiol concentrations in cryptorchid dogs (Cryptorchid) and dogs with tumour (Tumour). Obtained from peripheral blood (PB), local blood from right testicle (RT) and local blood from left testicle (LT). Number of measurements (n=8: Cryptorchid PB, LT, Tumour PB; n=8: Cryptorchid RT, Tumour LT; n=8 Tumour RT). Statistical significance ($p<0.05$): Tumour LT vs. Tumour RT, Tumour PB, Cryptorchid PB, Cryptorchid RT and Cryptorchid LT.

Table 1. Testosterone, anti-Mullerian hormone and oestradiol concentrations obtained from peripheral blood, local blood from right testicle and left testicle in intact dogs (Control), dogs with cryptorchidism (Cryptorchid) and dogs with tumour (Tumour). Data are expressed as arithmetic mean of measures (n=8) ± standard deviation.

	Testosterone (ng/ml)		
	Peripheral blood	Local blood right testicle	Local blood left testicle
Control	4.92 ± 3.3	46.23 ± 40.88	50.76 ± 43.76
Cryptorchid	0.89 ± 0.78	23.91 ± 22.79	10.52 ± 7.37
Tumour	11.37 ± 10.86	37.26 ± 25.26	44.86 ± 19.03
	AMH (μg/l)		
	Peripheral blood	Local blood right testicle	Local blood left testicle
Control	6.49 ± 3.24	6.25 ± 3.06	6.82 ± 3.09
Cryptorchid	54.98 ± 30.07	51.92 ± 30.59	46.33 ± 34.86
Tumour	28.13 ± 26.7	17.18 ± 12.5	23.9 ± 16.63
	Oestradiol (pg/ml)		
	Peripheral blood	Local blood right testicle	Local blood left testicle
Control	—	—	—
Cryptorchid	30.86 ± 20.28	55.71 ± 34.7	78.99 ± 47.72
Tumour	52.46 ± 34.02	188.16 ± 132.67	297.14 ± 245.56

Abbreviations: Anti-Müllerian hormone (AMH)

LT: 46.33 ± 34.86 μg/ml), ($p<0.05$) and slightly higher in the tumour group (RT: 17.18 ± 12.5 μg/ml; LT: 23.9 ± 16.63 μg/ml) in comparison with the control group (RT: 6.25 ± 3.06 μg/ml; LT: 6.82 ± 3.09 μg/ml) (Fig. 2, Table 1).

Oestradiol concentrations in dogs with cryptorchidism and tumour were high in the cryptorchid group in both peripheral and local blood (PB: 30.86 ± 20.28 pg/ml; RT: 55.71 ± 34.7 pg/ml; LT: 78.99 ± 47.72 pg/ml), and even higher in the tumour group (PB: 52.46 ± 34.02 pg/ml; RT: 188.16 ± 132.67 pg/ml; LT: 297.14 ± 245.56 pg/ml) (Fig. 3, Table 1).

In terms of testosterone, GnRH stimulation test was also performed. Figure 4 shows peripheral testosterone levels before and after the stimulation test in intact dogs (Control), dogs with cryptorchidism and dogs with tumour. A statistical significance ($p<0.05$) was found between testosterone concentrations before and after performing a stimulation test in all groups and there was shown the relevant increase in testosterone concentrations in all 3 groups.

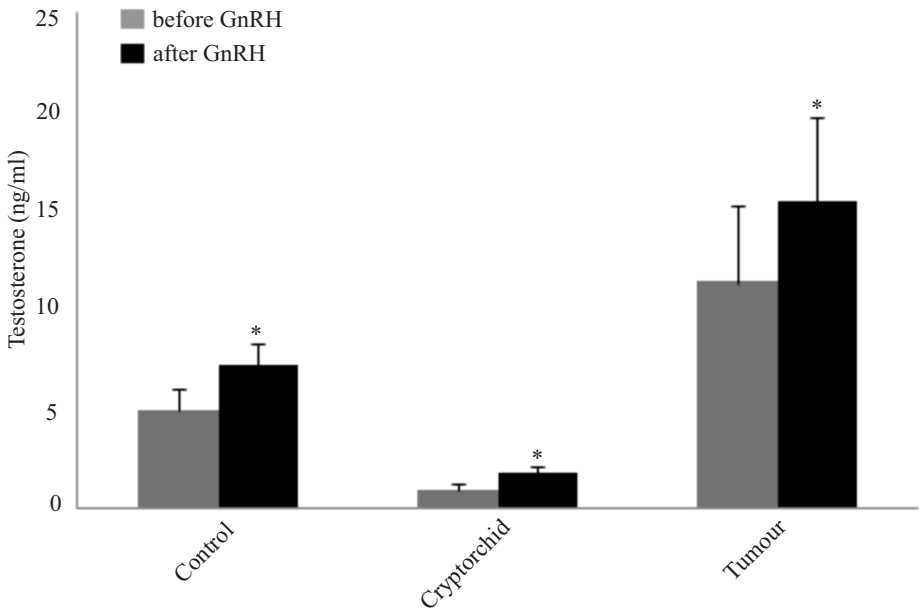

Fig. 4. Peripheral blood testosterone concentrations before and after performing a gonadotropin-releasing hormone stimulation test (GnRH) in healthy dogs (Control), dogs with cryptorchidism (Cryptorchid) and dogs with tumour (Tumour). Data are expressed as arithmetic mean of measures (n=8) ± standard error of mean. A statistical significance * ($p<0.05$) found between testosterone concentrations before and after performing a GnRH stimulation test in all groups.

No statistically significant differences were noted between the hormones concentration in left and right local testicular blood in the control group.

Discussion

The present study describes the levels of testosterone, anti-Mullerian hormone and 17β-oestradiol serum concentrations in peripheral and local spermatic venous blood in dogs with unilateral cryptorchidism, dogs with neoplastic diseases and in the control group of mature intact dogs.

It has been suggested that testosterone levels in peripheral and spermatic venous blood plasma in unilateral cryptorchid dogs do not differ from its levels in dogs with scrotal testes (Arteaga et al. 2000, Mischke et al. 2002). In contrast to the results of Mischke et al. (2002), all dogs in the cryptorchid group in this current study had a significant decrease of peripheral blood testosterone levels. We compared the secretory testosterone responses to GnRH application between dogs with scrotal testes, cryptorchid testes and testes with tumorous disease.

Due to the nature of the pulsatile secretion of testosterone during the day, it is recommended to measure and evaluate its concentration after the so-called stimulation test, when in healthy male dogs testosterone is released in 60 minutes after GnRH administration. By performing that we created the same conditions for determination of the maximum daily secretory peak of testosterone in all examined dogs. Testosterone concentrations were measured before and 60 minutes after performing a Gonadotropin-releasing hormone stimulation test with gonadorelin. This test is used to confirm the presence of abdominal cryptorchid testis while the increase of testosterone in the second sample clearly confirms its presence (De Gier et al. 2012). Testicular stimulation of normal, intact dogs with GnRH analogues produces a significant increase in plasma testosterone concentrations in response to subcutaneous or intramuscular application of GnRH (Kawakami et al. 1997, Ortega-Pacheco et al. 2006). This present study shows a significant increase of testosterone serum concentration after the GnRH applied intravenously in all groups of dogs, which is in agreement with previous reports on a significant increase in adult male dogs (Knol et al. 1993, De Gier et al. 2012). Romagnoli et al. (2017) suggested, that also in intact adult male cats, the intravenous application of 50 µg/cat is followed by a significant increase in serum testosterone output one hour later. Neoplastic cells within testicular tumors may produce estrogen and/or testosterone, resulting in excessive hormonal concentrations. High concentrations of testosterone have been reported in dogs with interstitial cell tumors and may lead to prostatic disease, perianal adenoma, perianal gland hyperplasia and perineal herniation (Johnston et al. 2001, Sanpera et al. 2002, Plavec et al. 2007, Grieco et al. 2008, Lopate 2010, Ciaputa et al. 2012). Indeed, the sustentacular cells of Sertoli contain large

amounts of estrogen, especially in the dog (Huggins and Moulder 1945). Hyperestrogenism leads to feminization of the male dog, which is observed in 25-50% of dogs with Sertoli cell tumors, in 5% of dogs with interstitial cell tumors, and uncommonly in cases with seminoma (Johnston et al. 2001, Mischke et al. 2002, Sanpera et al. 2002, Kim and Kim 2005, Lopate 2010). Hyperestrogenism leads to clinical signs, such as bilateral symmetrical alopecia, hyperpigmentation, gynecomastia, pendulous prepuce, linear preputial erythema, squamous metaplasia of the prostate gland and attraction of male dogs. Bone marrow hypoplasia, resulting in pancytopenia, may also be seen as a consequence of hyperestrogenism (Feldman and Nelson 2004, Lopate 2010). Oestradiol concentrations in healthy intact dogs are known to be under the detection limit in blood: <7 pg/ml (Mattheeuws and Comhaire 1989). De Gier et al. (2012) found that the administration of GnRH did not result in a significant increase in plasma oestradiol concentrations. A possible explanation for the lack of a significant increase in plasma oestradiol after GnRH in gonadally intact male dogs could be that oestradiol is aromatized from testosterone (De Gier et al. 2012). For cryptorchids in this study, mean oestradiol concentrations were higher to that of intact male dogs in Mischke et al. (2002) study, while the mean oestradiol concentration was 18.0 pg/ml. Oestradiol concentrations in dogs with cryptorchidism and tumour are high in the cryptorchid group in both peripheral and local blood (30.86 ± 20.28 pg/ml) and even higher in the tumour group (52.46 ± 34.02 pg/ml).

The aim of the present study was to determine the effectiveness of an AMH measurement as a diagnostic method for the presence of functional gonadal tissues in dogs. A negative correlation between testosterone and AMH has been found in human males (Rey et al. 1993) and it appears that testosterone suppresses AMH production in Sertoli cells (Boukari et al. 2009).

Concentration levels of anti-Mullerian hormone in peripheral blood were similar to concentration levels in local blood within each group. In contrast to findings in peripheral testosterone levels, the levels of testosterone in the control and the cryptorchid group between peripheral blood and local blood were considerably different. In the present study, intact male dogs had detectable AMH concentrations in peripheral blood (6.49 ± 3.24 µg/ml).

A higher concentration of AMH in intact dogs than in castrated dogs has been reported previously (Ano et al. 2014). Likewise, evaluating AMH concentrations in castrated, intact and cryptorchid stallions, Claes et al. (2013) found significantly higher concentrations of AMH in cryptorchid stallions than those in intact stallions. Moreover, measurement of serum AMH concentration has been indicated as a reliable method to determine the presence of testicular tissue in humans (Matuszczak et al. 2012). In the present study a significant increase in anti-Mullerian hormone concentrations in peripheral blood were observed in cryptorchid group (54.98 ± 30.07 µg/ml) compared to the control group ($p<0.05$). AMH profile is more efficient, sensitive and less time consuming than a plasma testosterone profile before and after an hCG stimulation or a GnRH test for evaluating the existence of a functional cryptorchid testis. Moreover, the AMH profile could be used as a confirmatory biomarker in supposedly castrated animals (Kitahara et al. 2012). Therefore, AMH may serve for determining of differential diagnosis of cryptorchidism from other conditions in which the testes are not present in the scrotum, including anorchism or previous castration (Gharagozlou et al. 2014, Holst and Dreimanis 2015).

Additionally, the concentrations of AMH in peripheral blood in tumour group (28.13 ± 26.7 µg/ml) were higher than in the control group, but the difference was not so significant. According to Banco et al. (2012) and Holst and Dreimanis (2015) AMH was not expressed by Leydig cells, spermatogonia, interstitium or epididymis in canine testes. In humans, AMH has been shown to be a specific marker of Sertoli cell presence in gonadal tumours (Rey et al. 2000). Using immunohistochemistry, AMH has been shown to be a useful marker of immature and neoplastic Sertoli cells in dogs (Banco et al. 2012). Due to this fact, according to Holst and Dreimanis (2015), it is possible to use the analysis of peripheral AMH level relevantly to differentiate diagnostic workup of dogs with suspected neoplastic testicular diseases in dogs. Anti-Müllerian hormone was demonstrated to be a promising biomarker for the diagnosis of canine Sertoli cell tumors (Ano et al. 2014).

In summary, a statistical significance ($p<0.05$) was found between testosterone concentrations before and after performing a GnRH stimulation test in all groups. A significant increase in anti-Mullerian hormone concentrations was found in the cryptorchid group in both, peripheral and local venous blood compared to the control one. We also observed high oestradiol concentrations in the cryptorchid and the tumour group.

In addition, in the present study a significantly lower peripheral testosterone levels were found in cryptorchid dogs group ($p<0.05$) in contrast to the tumour dogs group where a significantly higher peripheral testosterone concentration was found ($p<0.05$) compared to the healthy control group.

Taken together, our results show that the peripheral blood AMH concentration in cryptorchid group was approximately 9 times higher than in control group. Also the peripheral AMH concentration in tu-

mour group was approximately 4 times higher in comparison with the control group. When comparing the peripheral blood AMH concentration in tumour and cryptorchid group, nearly twice as low concentration in tumour group was present as in cryptorchid group.

The results of the present study show a possibility of using the peripheral blood levels of monitored hormones as a useful and quick test with a low invasivity to distinguish dogs with cryptorchidism and testicular tumours. The hormonal pattern for a cryptorchid dogs in our study: a significantly lower peripheral testosterone levels, a significantly higher peripheral AMH and oestradiol levels in comparison with peripheral hormones levels in control dogs. A significantly higher peripheral levels of testosterone, AMH and oestradiol were found in contrast to peripheral hormone levels in control group.

To the best of the authors' knowledge, present evaluation of testosterone, AMH and oestradiol levels in dogs with cryptorchidism and dogs with testicular tumour has not been reported in previous studies.

An absent histological typing of different kinds of testicular tumors is a certain limitation to this study. Therefore, it is not possible to evaluate the levels of studied hormones depending on the different types of testicular tumors. This fact offers the space for the further research in the form of a study aimed precisely to define the levels of selected hormones based on various types of testicular tumours.

Further studies might be needed to clarify the regulation of AMH secretion by Sertoli cells in the normal and cryptorchid testis and improve the precision of these findings.

Conclusion

In conclusion, the analysis of AMH is a valuable tool for the practitioner within small animal reproduction, and can, together with the analysis of steroids, give a better picture of pathological aberrations. AMH could be also used as a potential biomarker to distinguish castrated and cryptorchid dogs.

According to us, the scientific work dealing with a disorder of testicular descent in dogs, regarding the evaluation of sex hormones levels and the descent of the testes using modern diagnostic methods, significantly contribute to the clarification of some processes, leading to pathophysiological disturbances during this process.

Acknowledgements

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