Introduction

Castration is a common surgical procedure throughout the world as a means of population control among feral and domestic canine populations. Whilst the primary objective of a bilateral orchidectomy in dogs is to eliminate the breeding capability of the animal, the effect of the procedure has various effects on the internal hormonal environment within the animal. It is generally acknowledged that the primary male hormone testosterone declines in gonadectomised animals. Previous studies related to measuring blood testosterone concentrations in dogs have been conducted several weeks after the initial surgical procedure has occurred. The effect of the procedure on additional hormones levels has not been highly investigated, thus necessitating my investigation on this topic in this incidence.

Steroid hormones are commonly measured in dogs to detect a number of abnormal conditions. The most common of these includes determining if a male dog is castrated or a potential cryptorchid patient by measuring testosterone.
The primary male sexual steroid hormones are produced by the testicles. Additional to the gonadal tissue, the adrenal glands produce steroid intermediates, aldosterone, glucocorticoids and sex hormones in the cortex layers. The glucocorticoid, cortisol is a life-sustaining adrenal hormone, essential to maintain homeostasis. Referred to as the 'stress hormone' cortisol influences and regulates multiple changes in the body in response to stress including blood pressure, vascular tone and maintaining normo-glycaemia. Measuring steroid intermediates in veterinary medicine is applied during the diagnosis of Addison's and Cushing's disease.

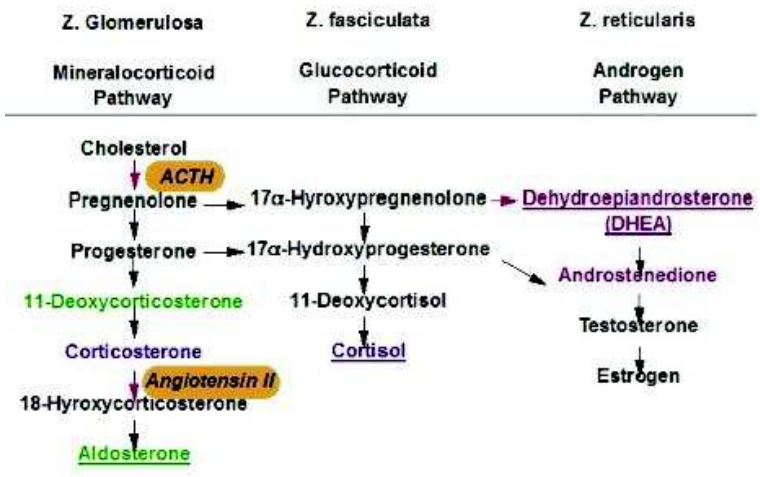
The aim of my thesis is to highlight the concentration profiles of various steroid hormones that were measured before and immediately post-operative, to try and give an insight into the internal compensatory mechanisms that occur during the orchidectomy surgical procedure in male dogs.

1. Literature Review

1.1 General overview of steroidogenesis:

It is universally acknowledged that steroids are a critical class of molecules in mammals. Steroids regulate a wide range of physiological functions such as reproduction, blood-salt balance, and response to stress, neuronal function, metabolic processes (fat, muscle, bone formation) and have a key role as endocrine signalling molecule (Ruiz-Cortés, 2012).There are five major steroid hormone groups: androgens (testosterone), oestrogens, progestogens (progesterone), glucocorticoids (cortisol/corticosterone) and mineralocorticoids (aldosterone). Steroids exert physiological responses "through their binding to nuclear and extracellular receptors, eliciting complex intracellular signalling and genetic transcriptional responses" (Midzak & Papadopoulos, 2015). Steroidogenesis is the biological process of steroid production. All steroid hormones are generated from the parent compound cholesterol through a series of enzymatic steps in the mitochondria and endoplasmic reticulum of steroidogenic tissues (figure 1). Such steroidogenic tissues includes the adrenal gland, testis, ovaries, adipose tissue and brain. There are multiple sources of cholesterol for steroid production: a) cholesterol synthesis de novo from acetate, b) cholesterol obtained from plasma low density lipoproteins (LDL) and high density lipoproteins (HDL), c) hydrolysis of stored cellular cholesterol esters in intracellular lipid droplets. The steroid classes share a common intermediary steroid pregnenolone. Regulation of steroidogenesis involves control of enzymes-cytochrome P450 (CYP) and hydroxysteroid dehydrogenase (HSD) proteins that modify cholesterol into the steroid hormone of interest.

Figure 1: Major Pathway in Steroid Biosynthesis



Source: Weiss (2015)

1.2 The Sexual Steroids

The sexual steroids, testosterone, oestrogen and progesterone are necessary for gonadal development and function. They influence sexual differentiation, control secondary sexual characteristics and sexual behaviour (Modesto et al., 2015). "Steroidogenesis of gonadal sex hormones is by definition sexually dimorphic in hormonal action and also in regulation and temporal patterns of production" (Ruiz-Cortés., 2012). In male animals, the primary sexual steroid hormone is testosterone, whilst in female animals oestrogen is predominant.

1.3 Testosterone

1.3.1 Source of Testosterone

Testosterone is an androgenic anabolic steroid hormone, produced primarily in the Leydig cells of the testes, and to a lesser extent by the adrenal gland cortex. Testosterone production in males is regulated by two Leydig cell populations, foetal and adult Leydig cells (Tremblay., 2015). In-utero a DNA-binding protein 'Testis Determining Factor' (TDF) encoded by the 'sex determining region Y' gene (SRY) found on the Y chromosome is responsible for the determination of the male sex in the foetus (MCELREAVEY et al., 1993). The SRY gene promotes Sertoli cell differentiation from tubular cells of the embryonic gonadal ridge that originated from the adrenal-gonadal primordium. Sertoli cells secrete Anti-Mullerian Factor which inhibits development of female reproductive organs and induces surrounding neighbouring cells to differentiate into leydig cells, the source of foetal testosterone (Modesto et al., 2015).

1.3.2 Role of Testosterone

Foetal testosterone influences in utero development of the testes, epididymis, vas deferens, the external male genitalia (penis and scrotum) and accessory sex glands; whilst influences testicular descent in puppies.

In juvenile and adult dogs, testosterone has a primary role in the development and function of male reproductive tissues, maintains spermatogenesis, and promotes the development of the secondary male sexual characteristics; hair growth, increase in muscle and bone mass.

It has also been noted that testosterone has beneficial effects on the cardiovascular system (Chou et al., 1996). According to research, there are variations in testosterone levels in accordance with age. Plasma testosterone concentrations begin to rise in pre-pubertal dogs aged 4-6 months, peaking at puberty, dogs aged 6-12 months, eventually plateau's until gradually declining in geriatric animals.

Testosterone is responsible for the display of male sexual behaviour and is associated with aggression in dogs. A study in male rats concluded that certain aspects of male sexual behaviour is androgen dependent, most notably intromission frequency and post-ejaculatory intervals (Damassa et al., 1977). "In male dogs, testosterone influences changes in the basolateral nuclear group of the amygdaloid body, increasing the number of androgen receptor neurons associated with pathological aggression in dogs" (Jacobs et al., (2006).

1.3.3 Testosterone Production and Control

The first step of testosterone biosynthesis is the oxidative cleavage of the cholesterol sidechain by mitochondrial cytochrome P450 oxidase, losing six carbon atoms to yield pregnenolone. An additional removal of two carbon atoms in the endoplasmic reticulum by CYP17A enzyme produces the C19 steroids. Oxidation of the 3-hydroxyl group by 3-β-HSD produces androstenedione. The final step is the reduction of androstenedione by 17-β hydroxysteroid dehydrogenase to yield testosterone (see figure 1).

Testicular testosterone production is controlled by the hypothalamus-pituitary-gonadal axis. Low plasma testosterone stimulates hypothalamic gonadotropin releasing hormone (GnRH). GnRH stimulates pituitary production of luteinising hormone (LH). Luteinizing hormone is released into the blood, stimulating testosterone production and release in the gonads. High blood testosterone levels inhibit GnRH release from the hypothalamus via negative feedback.

1.3.4 Abnormal Testosterone Concentrations

The normal testosterone range is 0.1-0.94ng/ml (Pathirana et al., 2012), with seasonal fluctuations having been observed, found to be highest in autumn and spring (Martins et al., (2006).

Low testosterone levels are observed in neutered animals, Klinefelter's Syndrome (Reimann-Berg et al., 2008), Sertoli cell tumours (Peters et al., 2000), cryptorchidism (Eik-Nes., 1966) and hypogonadism (Ortega-Pacheco et al., 2006), consequentially diminishing libido and contributing to poor sperm quality and infertility.

Excessively high levels of testosterone are observed in hyperadrenocorticism (Hill et al., 2005) and hyperandrogenism. Hyperandrogenism is a rare condition, most frequent in intact male dogs. The condition is characterized by elevation of masculinizing sex hormones in the blood, such as testosterone and its derivative di-hydrotestosterone (DHT). The elevated blood hormone concentration can manifest due to excessive hormone production by the testes, ovaries and the adrenal cortex. Clinical manifestation may include behavioural changes, abnormalities in the reproductive tract or dermatological issues.

1.4 Di-hydrotestosterone

1.4.1 Source of DHT

Di-hydrotestosterone is an androgenic hormone and metabolite converted from testosterone. Blood DHT levels are regulated by testosterone, low testosterone levels stimulates pulsatile release of GnRH from the hypothalamus which in turn stimulates the secretion of pituitary LH and follicle stimulating hormone (FSH) into blood circulation. LH stimulates the production and release of testosterone from the Leydig cells. Testosterone is then converted into DHT in target tissues by 5α-reductase enzyme. The site of DHT production includes the testes, prostate gland, ovaries, skin and adrenal glands (Abdel-Rahman., 2015; Gloyna & Wilson., 1969).

1.4.2 Role of DHT

Circulating DHT is less abundant than testosterone, although it does account for most of testosterone's biological action. Like testosterone, DHT exerts its physiological effect via androgen-receptors found in cellular DNA. DHT activates the gene expression responsible for primary and secondary male sexual development and behaviour (Romeo et al., 2001).

DHT is important during embryogenesis (Deslypere et al., 1992), particularly from day 36. Testicular tissue present in utero, produces testosterone, from which DHT is produced. DHT has an essential role in the formation of the male external genitalia; closure of the urethra, prostate and scrotum.

1.4.3 Abnormal DHT concentrations

DHT is the biological active hormone that promotes Benign Prostatic Hyperplasia (BPH). BPH is common in intact males at 6+ years. BPH manifestation can be a result of androgen stimulation or altered androgen: oestrogen ratio. Elevated oestrogen levels results in increased oestrogen receptors on the prostate and prostatic urethra. DHT binds to the androgen receptors, stimulating cellular growth and division (Wilson., 1980).

1.5 Oestrogen

1.5.1 Source of Oestrogen

Endogenous oestrogen is a steroid hormone produced primarily by the granulosa cell of tertiary follicles in the ovaries of female mammals, placenta of pregnant animals and to a lesser extent in the testicles and adrenal cortex (Santen et al., 1980). Additional sites of synthesis includes the liver, muscle, adipose tissue and hair follicles. There are three oestrogen compounds: oestrone, oestradiol and oestriol. In mammals, the main oestrogen type produced is oestradiol. A study related to blood oestradiol concentration in normal neutered female and male dogs discovered oestradiol concentrations ranging between 12.5-53.5pg/mL (Frank et al., 2010).

1.5.2 Role of Oestrogen

Oestrogen has multiple neuroendocrine functions in the body including normal growth and development of the female reproductive tract, development of secondary sexual characteristics, mammary gland development and body conformation. Oestrogen influences the clinical onset and sexual receptivity in females during oestrus. Other physiological functions of oestrogen includes, increasing blood coagulation, fluid balance, bone formation and maintenance of blood vessels and skin (Hasan et al., 2009). Oestradiol has been associated with neuroprotective properties. Injury to the brain, induces astroglia to express aromatase enzymes which catalyse testosterone, pregnenolone and other C19 steroids transformation into oestradiol, to cope with the neurodegenerative effects caused by injury (Garcia-Segura et al. (2003).

1.5.3 Oestrogen Production

In females, enzymatic aromatase pathways synthesize oestrogen from either testosterone or androstenedione which are produced by theca cells in the follicle (Ruiz-Cortés., 2012). Oestrogen is transported in the blood bound to a sex hormone binding globulin (SHBG) and to albumin at a lower concentration. In male animals, testosterone can be converted to oestradiol by an aromatizing enzyme that is found in the testes, prostate and bone, thus contributing to the peripheral oestradiol levels found in the male dog (Rijnberk., 2010).

1.5.4 Oestrogen in male dogs

While Oestrogen is considered the primary female sex hormone, it has been discovered that it plays an essential role in male fertility. Peripheral target tissues contain aromatase enzyme that facilitates the conversion of circulating testosterone to oestradiol and androstendione to oestrone. Aromatase expression in males includes the Leydig cells, Sertoli cells, adipose tissue, bone osteoblasts and chondrocytes.

Oestrogen is produced by Leydig cells in the adult male testes, thus there is a high concentration in the rete testis fluid. Studies in mice have discovered that oestrogen receptors have been located in the testes, efferent ductules and epididymis (Nie et al., 2002; Zhous et al., 2002). The receptors regulate protein expression required for fluid resorption. Alterations to the receptors results in sperm dilution in the cauda epididymis, altered sperm morphology and disruption in water and sodium resorption, eventually resulting in decreased fertility in males (Hess., 2003).

1.5.5 Abnormal Oestrogen Concentrations

Excess oestrogen production 'hyper-oestrogenism' has been observed in both male and female dogs. The condition can manifest due to granulosa cell tumours or cystic ovaries in females (Ghaffari et al., 2009), whilst in males it can occur due to Sertoli cell tumours (Lew el at., 2005). Clinical manifestation of hyper-oestrogenism includes feminisation, enlarged mammary glands and teats in both sexes, pendulous prepuce and penile atrophy in males, and symmetrical alopecia and hyper-pigmentation.

Elevated oestrogen levels has also been reported to affect the bone marrow, causing irreversible pancytopenia or aplastic anaemia (Sherding et al., 1981; Hasan et al., 2009).

1.6 Progesterone

1.6.1 Source and Role of Progesterone

Progesterone (P4) is an endogenous steroid and progestogen sex hormone, secreted by the placenta of some species, the ovarian corpus luteum in females (Meyer., 1994) and adrenal glands. Additionally it is also produced in nervous and adipose tissues and the male testes.

P4 is a crucial intermediate adrenal metabolite involved in the production pathway of other endogenous steroids, the corticosteroids, mineralocorticoids and androgens. In females, P4 prepares the uterus for nidation, promotes endometrial gland proliferation and pregnancy

maintenance. Progesterone levels fluctuate dependant on stage of the oestrus cycle in females. In the dog, the P4 slowly increases during pro-oestrus, from basal values of 0.2-0.4ng/ml. With the onset of a LH surge, there is a dramatic increase of progesterone levels, consequentially, ovulation occurs when progesterone levels reach between 4.0-12.0 ng/ml (Concannon et al., 2009). Surprisingly P4 has an important role as in brain functions as a neuro-steroid (Baulieu & Schumacher., 2000).

1.6.2 Progesterone Production

Progesterone is synthesized from pregnenolone which is derived from cholesterol. There are two steps involved in the conversion of pregnenolone to progesterone.

1.7 Adrenal steroid production

The adrenal glands are paired bodies located immediately cranial to the kidneys in the retroperitoneal space in the abdominal cavity. The gland is comprised of the adrenal cortex and medulla. Steroids are produced in the three layers of the adrenal cortex, mineralocorticoids in the outer most layer the zona glomerulosa, glucocorticoids in the zona fasciculate and androgens in the zona reticularis. The first enzymatic step is the conversion of cholesterol to pregnenolone in the mitochondria. The reaction is carried out by the enzyme, cytochrome P450 side-chain cleavage. It is a rate limiting, irreversible step in the initiation of steroid synthesis that occurs in the adrenal, ovaries and testes.

In the adrenal gland, pregnenolone is then converted into three different pathways regulated by specific enzymes, dependent on the steroid in question that is being produced.

1.8 Cortisol

1.8.1 Source of Cortisol

Cortisol is a steroid hormone belonging to the glucocorticoid class. It is produced in the zona fasciculata in the cortex of the adrenal glands.

1.8.2 Cortisol Production and Control

In the adrenal gland, cortisol is synthesized from the precursor molecule cholesterol via pregnenolone and progesterone through a series of hydroxylation steps, catalysed by cytochrome P450-dependent hydroxylases (Denwick., n.d.). Approximately 67-87% of cortisol in blood is bound to a "specific α1-glycoprotein, corticosteroid-binding globulin (CBG)", 7-19% of cortisol is bound to albumin, whilst free-cortisol is 6-14% (Gayrard et al., 1996). Only the free-cortisol fraction is biologically active in dogs.

Regulation of cortisol release is controlled via the hypothalamic-pituitary-adrenal gland axis. Environmental or internal stimuli, such as stress or disease, stimulates the release of corticotropin-releasing hormone (CRH), a polypeptide consisting of 41 amino acid residues,

from the hypothalamus. CRH triggers a release of adrenocorticotrophic hormone (ACTH), a 39 amino acid peptide, from the anterior pituitary gland into the bloodstream. ACTH travels in the blood to the adrenal cortex, resulting in the synthesis and release of cortisol. Increased cortisol levels inhibits ACTH release via negative feedback (Desborough., 2000). In dogs, circadian rhythm for cortisol secretion is absent, studies have discovered that there was no variation in cortisol concentrations measured hourly over a ten hour period (Kemppainen & Sertin., 1984; Pessina et al., 2009). Normal pre-ACTH cortisol levels in dogs ranges from 13.8-137.9 nmol/L (Klein & Peterson., 2010).

1.8.3 Role of Cortisol

Cortisol is referred to as the stress-hormone, and is released in response to stress to help maintain an internal homeostasis (Villiers et al., 1997). Functions of cortisol includes, maintaining blood pressure, water balance and vascular volume, it increases blood sugar levels by promoting hepatic gluconeogenesis, aids in metabolism of proteins and carbohydrates and promotes lipolysis to maintain normo-glycemia in the fasting animal, it decreases peripheral cellular utilization of glucose, acts as an immunosuppressant, counteracts stress and has anti-inflammatory activities (Klein & Peterson., 2010).

1.8.4 Cushing's Syndrome

Hyperadrenocorticisim is a common endocrine disorder of dogs aged 6 years or more. There are three forms, pituitary-dependent hyperadrenocorticisim (PDH), adrenal-dependent hyperadrenocorticisim and iatrogenic hyperadrenocorticisim. Of the three forms, PDH is the most frequent, accounting for 85-90% of the cases (Peterson., 2001). Over 90% of PDH cases in dogs are a consequence of a pituitary tumour, resulting in bilateral adrenal hyperplasia and increased glucocorticoid release. Common clinical manifestation in the patient includes polyuria, polydipsia, alopecia, thin skin, obesity, hepatomegaly, and increased appetite. Laboratory results usually demonstrate eosinophilia, lymphopenia, elevated alkaline phosphatase and increased serum cortisol concentration.

1.8.5 Addison Disease

Hypoadrenocorticisim is an uncommon canine disease, typically observed in young to middle-aged dogs. Manifestation of Addison disease can occur due to immune-mediated destruction of the adrenal cortex layers leading to inadequate levels of mineralocorticoid and glucocorticoid steroids (primary adrenocortical insufficiency) or due to decreased ACTH production leading to a deficiency in cortisol production (secondary adrenocortical insufficiency). Primary adrenocortical insufficiency is characterized by hyperkalemia and hyponatremia due to decreased aldosterone regulation of sodium, potassium and water homeostasis (Machida et al., 2008). Clinical manifestation in patients includes vomiting, diarrhoea, dehydration, anorexia, weight loss and lethargy. Consistent serum chemistry abnormalities among dogs with hypoadrenocorticisim, particularly primary hypoadrenocorticisim are hyperkalemia, hyponatremia, and hypochloremia, elevated blood

urea nitrogen (BUN) values as a consequence of decreased renal perfusion resulting in pre-renal azotemia.

1.9 Corticosterone

Source and Production of Corticosterone

Corticosterone is a 21-carbon steroid hormone produced in the zona glomerulosa of the adrenal cortex. The adrenal gland has an abundant amount of receptors that utilise low density lipoproteins (LDL). Cholesterol, the precursor molecule involved in steroidogenesis, is liberated from the LDL molecules, and is the primary precursor molecule for glucocorticoid, mineralocorticoid and androgen synthesis. Corticosterone is an important intermediary step in the steroidogenic mineralocorticoid path. The mitochondrial bound haemoprotein cytochrome P-450 aldosterone synthase converts 11-deoxycorticosterone via corticosterone to the mineralocorticoid aldosterone in the zona glomerulosa of the adrenal cortex. Aldosterone is a major homeostatic modulator of potassium, sodium and water in the body and has a central role in regulating blood pressure (Rijnberk., 2010). Like cortisol, corticosterone is predominantly bound to corticosteroid-binding globulin plasma proteins in the blood at a lower affinity (Machida et al., 2008).

1.10 A Review on Gonadectomy

Gonadectomy or 'neutering' is one of the most common elective procedures performed by veterinarians. This elective surgery refers to the removal of the gonads, -testicles, ovaries and/or uterus. It is estimated that there is over 500 million dogs worldwide. Historically, the primary intention of a gonadectomy, i.e ovariohysterectomy (OHE), ovarioectomy in females and orchidectomy in males, was to sterilize the animal, today gonadectomies are performed as a mean of population control measures among stray, feral and domestic animals, and has numerous health benefits in both cases (Jackman & Rowan,. 2007).

In dogs, the traditional age to undergo a gonadectomy is 6-9 months old. Indications for the elective procedure may originate due to behavioural or medical recommendation. Gonadectomies performed at 6-14 weeks old may affect growth development and general health of the individual patient, but there is no significant changes compared to an animal that was neutered at a later age.

There is a mixture of benefits and adverse effects associated with this elective procedure dependent upon the age at neutering, sex and breed.

Frequent benefits associated with neutering are most notably behavioural. Behavioural changes commonly observed includes: decreased aggression, altered sexual dimorphic behaviour such as mounting and urine spraying, spayed bitches do not attract male dogs, and castrated males are less likely to roam looking for females in oestrus. Sterilizing an animal also eliminates passing on undesirable genetic traits and genes to canine offspring.

The health benefits of sterilization in females are considerable. Mammary neoplasia in female dogs are a common occurrence, with a reported incidence of 3.4%. More than 50% of the cases are reported as malignant. Sexually intact females are seven times more vulnerable to developing mammary neoplasia than spayed females. It has been reported, that if a female is spayed before her first heat, there is a 0.5% risk of mammary neoplasia occurring at a later age, an 8% risk of mammary neoplasia occurring after their first heat, and 26% risk of occurrence after their second heat. Ovariohysterectomy (OHE) is recommended in females as it also prevents the pyometra, and the occurrence of ovarian and uterine neoplasia in bitches.

Castration in males decreases circulating levels of testosterone, causing prostatic atrophy, thus reducing the incidence of benign prostatic hyperplasia (BPH), prostatitis and testicular neoplasia's. Clinical evidence of BPH occurring in intact males at 6+ years old is reported to be 75-80%, while BPH occurrence in males greater than 9 years old is 95-100% (Decramer & May., 2015).

Testicular neoplasia are common in intact male dogs, the incidence of testicular neoplasia in male dogs is 4.6% (Mischke et al., 2002). Sertoli cell tumours, interstitial cell tumours and seminomas are the most frequently reported testicular tumours. The ethology of testicular tumour development is unknown. Cryptorchid males with either uni- or bi-lateral undescended testicles are thirteen times more likely to develop a tumour in comparison to a

dog with normal scrotal testicles. Testicular tumours are slow to metastasize, the most common site of spreading is the regional lymph nodes, liver and lungs. The occurrence of testicular neoplasia in dogs can be prevented with routine castrations (Degner., n.d.).

There are numerous adverse side effects that may occur after a gonadectomy procedure. In females, post-operative complications may include ovarian remnant syndrome, stump pyometra, haemorrhages, and accidental ligation of the intestine or urethers.

A common sequel to sterilization in bitches is cystitis and urinary incontinence (UI). UI can develop within days of the surgery or several years later. "Females gonadectomised before 3 months of age appear to be at highest risk, compared with those gonadectomised at ≥ 3 months of age" (Spain et al., 2004). Although any size bitch can be affected, certain breeds are associated with UI development including Dobermans, Springer Spaniels, Irish Setters and Rottweilers. The theory behind UI development in spayed bitches is that due to the high proportion of collagen in spayed bitches compared to intact females, there is a loss of elasticity. This loss has adverse effects on bladder contractility and micturition (Rijnberk., 2010).

In male dogs, the reported incidence of prostatic neoplasms is 0.2-0.6% and are almost always malignant adenocarcinomas. Castrated males are at an increased risk for development, ranging from 2-4 times than that of intact males. A common neoplasm of the urinary tract is the transitional cell carcinoma (TCC) of the bladder. Breed variations at risk of TCC occurrence includes Beagles, Collies, West Highland Terriers and Wire Fox Terriers. Gonadectomised animals are at a greater risk of TCC development compared to intact animals.

The occurrence of obesity and related orthopaedic sequels exists in gonadectomised animals. In a study observing obesity in intact dogs compared to gonadectomised animals, 32% of the spayed bitches and 32% of the sterilized males were obese (De Cramer & May., 2015). Rupture of the cranial cruciate ligament (CCL) has been noted in sterilized dogs. A reported incidence of CCL rupture is 1.8%, and is more prevalent in gonadectomised animals, due to sexual steroid deprivation and continuously high plasma gonadotropin concentrations which in turn affect muscles and collagen.

2. Materials and Methods

2.1 Animals

A selection of male dogs participating in an on-going sterilization programme currently being conducted in Szent Istvan University, Faculty of Veterinary Science were used as candidates for the study. The animals participating in the programme were from a local animal rescue centre. The dogs arrived early in the morning, on the day of surgery and were housed in crates located in the Obstetrics department at the small animal clinic on campus.

2.2 Sample collection and analysis

Blood and tissue samples were obtained from 20 male dogs. Two blood samples were drawn from the cephalic vein on the day of the scheduled surgery from each individual dog both prior to and after the castration. The left and right testicular tissue was collected during the orchidectomy, for additional tissue measurement of progesterone, testosterone and di-hydrotestosterone levels. The blood samples were collected in tubes without anticoagulant and serum was separated by centrifugation 3000g. Plasma progesterone was measured by an enzyme immunoassay (ELISA) validated for the dog (Quanticheck, Budapest). The serum testosterone, 17β-oestradiol, pregnenolone, corticosterone, cortisol, 17-α-hydroxyprogesterone and di-hydrotestosterone were measured by ELISA (DRG International, USA).

2.3 Anaesthesia

The dogs were sedated using Ketamine (10-15mg/bw) and xylazine (1mg/bw). Isoflurane was used for maintenance during the surgery.

2.4 Statistical Analysis

'SPSS' version 19.0 for Windows was used to analyse the data collected. Paired T-tests were used to measure changes in hormone levels from blood and testicular tissue samples collected pre and post-castration. The results were considered significant if $P < 0.05$.

3. Results

Cortisol, corticosterone and 17-alpha-hydroxyprogesterone had increased significantly. There was an obvious decrease in testosterone and DHT values when measured post-castration.

The mean corticosterone value prior to surgery was 108.36 nmol/L, after the orchidectomy, corticosterone values had increased to 192.79 nmol/L (figure 4). Cortisol values after the orchidectomy had also increased, prior to the surgery, mean cortisol levels were 134.99 nmol/L, whilst post-surgery they had increased to 234.16 nmol/L (figure 5). Mean values for 17-α -hydroxyprogesterone prior to the orchidectomy were 0.98 ng/ml. The mean value had increased in the animal when the hormone was measured again post-castration to 1.61 ng/ml (figure 9).

Testosterone and DHT decreased post-castration. The mean testosterone values prior to surgery was 13.25 ng/ml, whilst when measured again post-castration, the mean value had decreased to 5.39 ng/ml (figure 7). Mean value for DHT prior to the surgery was 3335.01 pg/ml. After the orchidectomy, the mean value had decreased to 2076.30 pg/ml (figure 8).

The remaining hormones, progesterone, pregnenolone and oestrogen showed no significant changes in blood concentrations when measured (figures 2, 3 5).

Table 1: Concentration of Progesterone

	Number of samples	Mean pre-castration	Mean post-castration	Mean	T-Value	DF	Sig.
P4 ng/ml	20	4.04 ±1.936	2.89 ±0.527	1.15 ±2.047	0.560	19	0.582

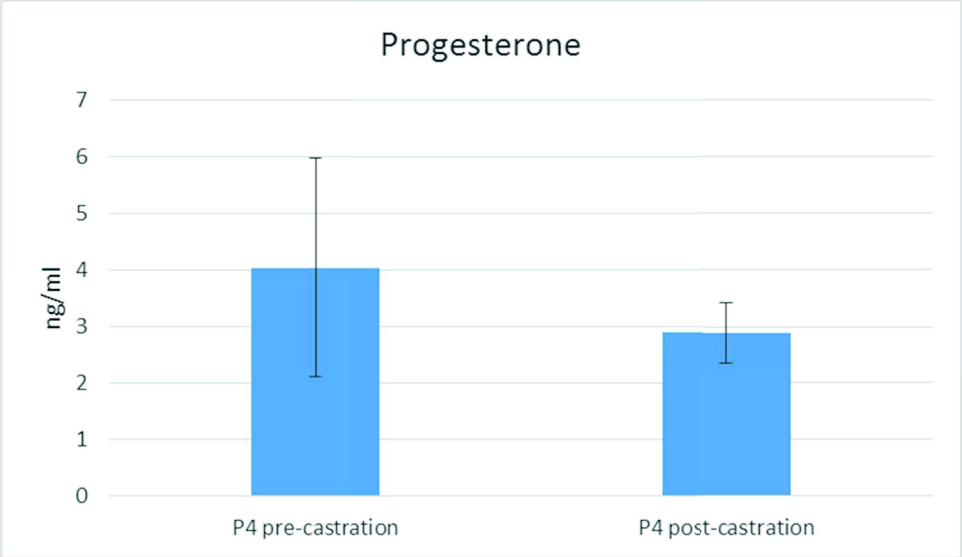


Figure 2: Concentration of Progesterone

There was no significant difference in serum progesterone concentrations measured pre- and post-castration (Mean=1.15 ±2.047, T=0.560, DF=19, P=0.582).

Table 2: Concentration of Pregnenolone

	Number of samples	Mean pre-castration	Mean post-castration	Mean	T-Value	DF	Sig.
Pregnenolone ng/ml	**19**	**28.14 ±5.088**	**26.88 ±5.024**	**1.26 ±2.371**	**0.529**	**18**	**0.603**

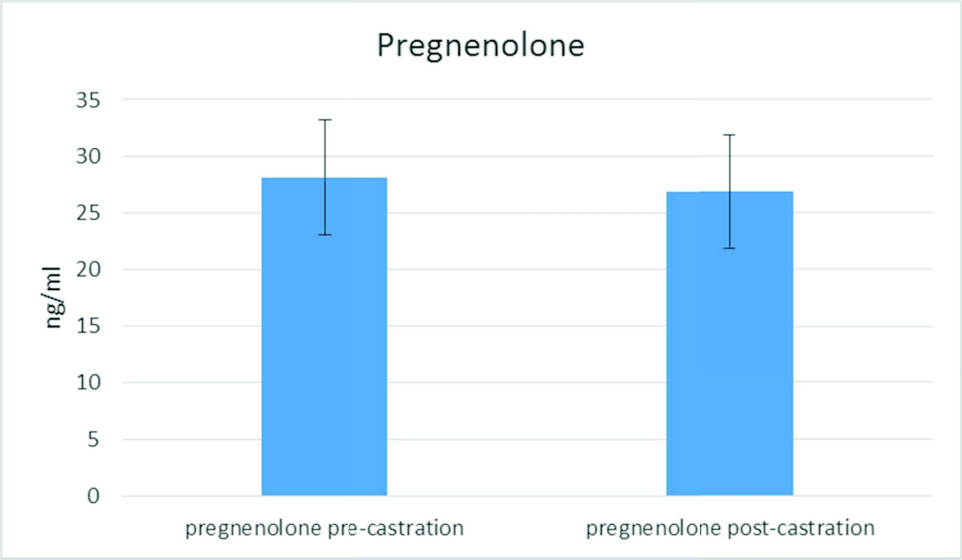


Figure 3: Concentration of Pregnenolone

There was no significant difference in Pregnenolone values pre and post-castration (Mean=1.26 ±2.371, T=0.529, DF=18, P=0.603).

Table 3: Concentration of Corticosterone

	Number of samples	Mean pre-castration	Mean post-castration	Mean	T-Value	DF	Sig.
Corticosterone nmol/l	**20**	**108.36 ±25.346**	**192.79 ±37.331**	**-84.43 ±24.891**	**-3.392**	**19**	**0.003**

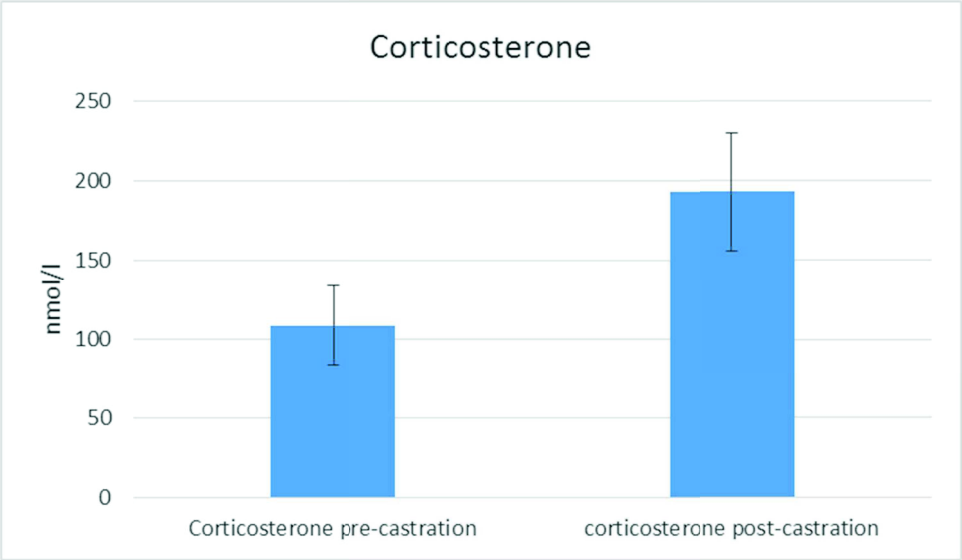


Figure 4: Concentration of Corticosterone

There was a significant difference in Corticosterone values pre and post-castration (Mean= -84.43 ±24.891, T= -3.392, DF=19, P=0.003).

Table 4: Concentration of Cortisol

	Number of samples	Mean pre-castration	Mean post-castration	Mean	T-Value	DF	Sig.
cortisol nmol/l	**20**	**137.99 ±23.363**	**234.16 ±36.868**	**-99.16 ±33.720**	**-2.941**	**19**	**0.008**

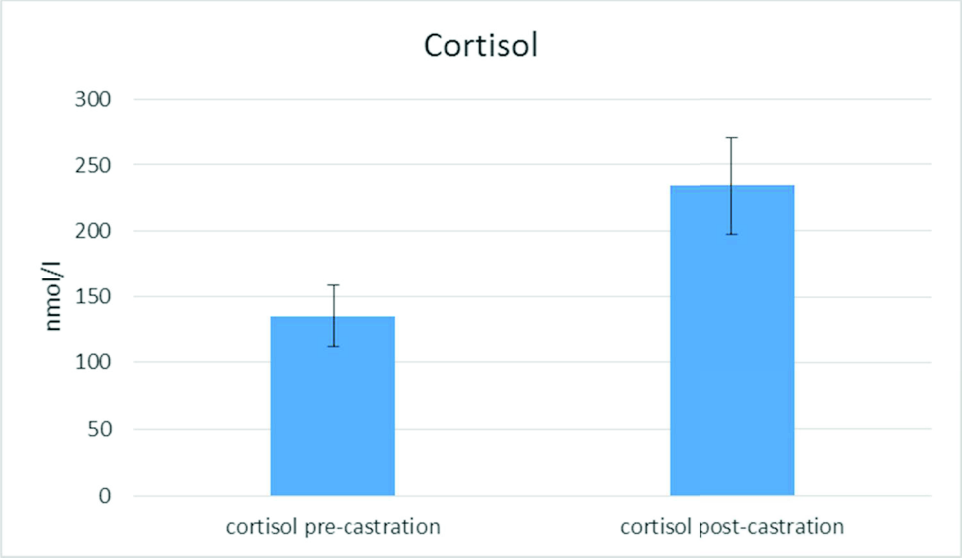


Figure 5: Concentration of Cortisol

There was a significant difference in Cortisol values pre and post-castration (Mean=-99.16 ±33.720, T=-2.941, DF=19, P=0.008).

Table 5: Concentration of Oestrogen

	Number of samples	Mean pre-castration	Mean post-castration	Mean	T-Value	DF	Sig.
Sensitive E2 pg/ml	**20**	**65.08 ±13.348**	**54.59 ±15.184**	**10.48 ±8.808**	**1.190**	**19**	**0.249**

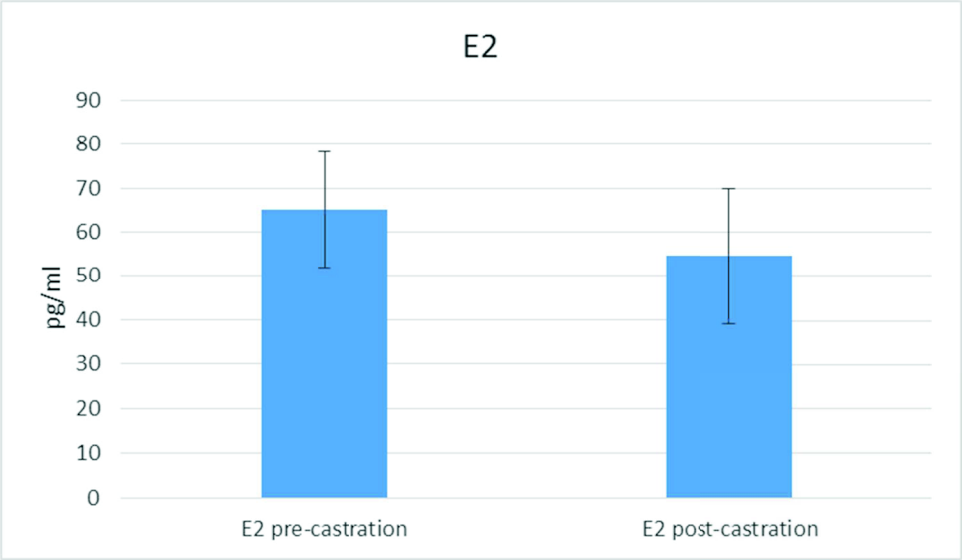


Figure 6: Concentration of Oestrogen

There was no significant difference in Oestrogen values pre and post-castration (Mean=10.48 ±8.808, T=1.190, DF=19, P=0.249).

Table 6: Concentration of Testosterone

	Number of samples	Mean pre-castration	Mean post-castration	Mean	T-Value	DF	Sig.
Testosterone ng/ml	**20**	**13.25 ±2.890**	**5.39 ±1.563**	**7.86 ±1.745**	**4.504**	**19**	**<0.001**

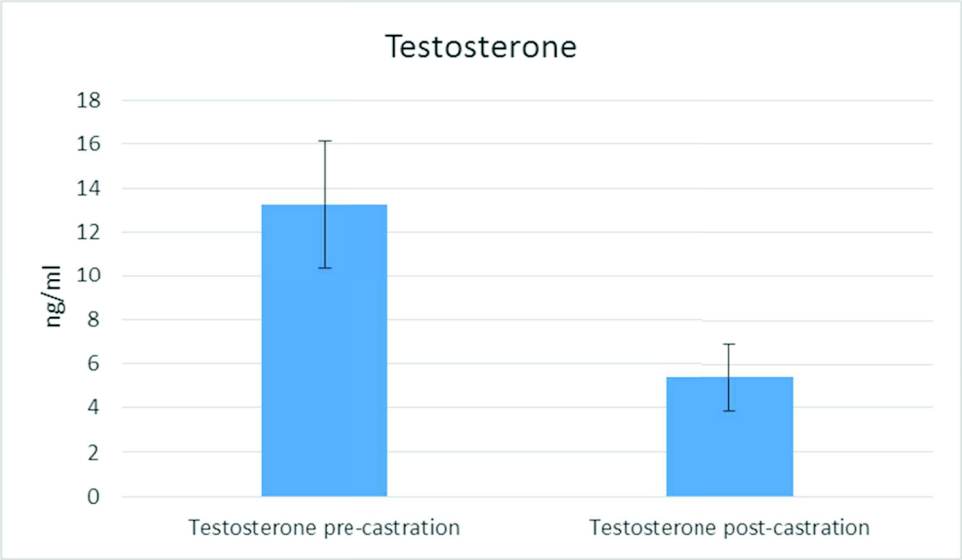


Figure 7: Concentration of Testosterone

There was a highly significant difference in Testosterone values pre and post-castration (Mean=7.84 ±1.745, T=4.504, DF=19, P <0.001).

Table 7: Concentration of Di-hydrotestosterone

	Number of samples	Mean pre-castration	Mean post-castration	Mean	T-Value	DF	Sig.
DHT pg/ml	**20**	**3335.01 ±473.940**	**2076.30 ±380.925**	**1258.71 ±267.401**	**4.707**	**19**	**<0.001**

*Di-hydrotestosterone

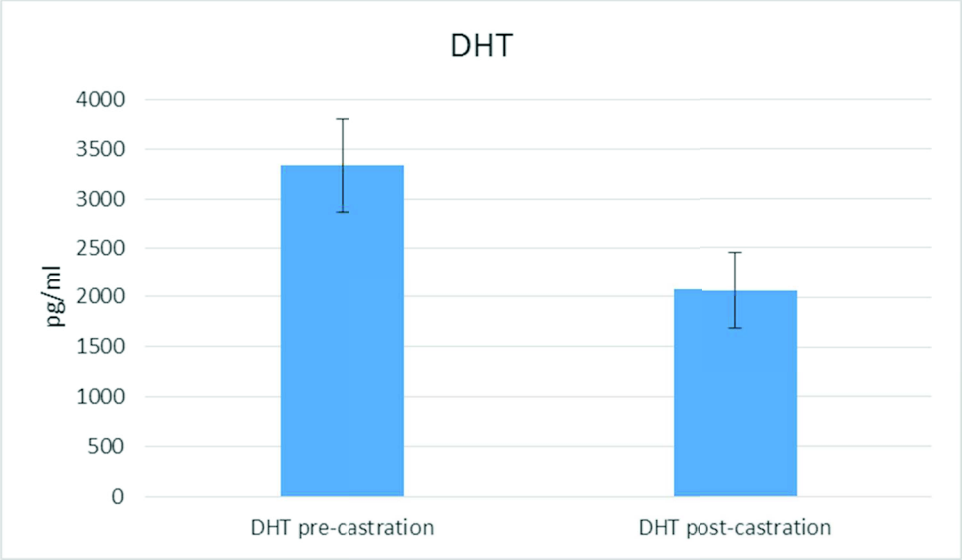


Figure 8: Concentration of Di-hydrotestosterone

There was a highly significant difference in di-hydrotestosterone values pre and post-castration (Mean=1258.71 ±267.401, T=4.707, DF=19, P <0.001).

Table 8: Concentration of 17-α-hydroxyprogesterone

	Number of samples	Mean pre-castration	Mean post-castration	Mean	T-Value	DF	Sig.
17-α-OH P4* ng/ml	19	0.98 ±0.207	1.61 ±0.338	-0.63 ±0.297	-2.132	18	0.047

*17-α-hydroxyprogesterone

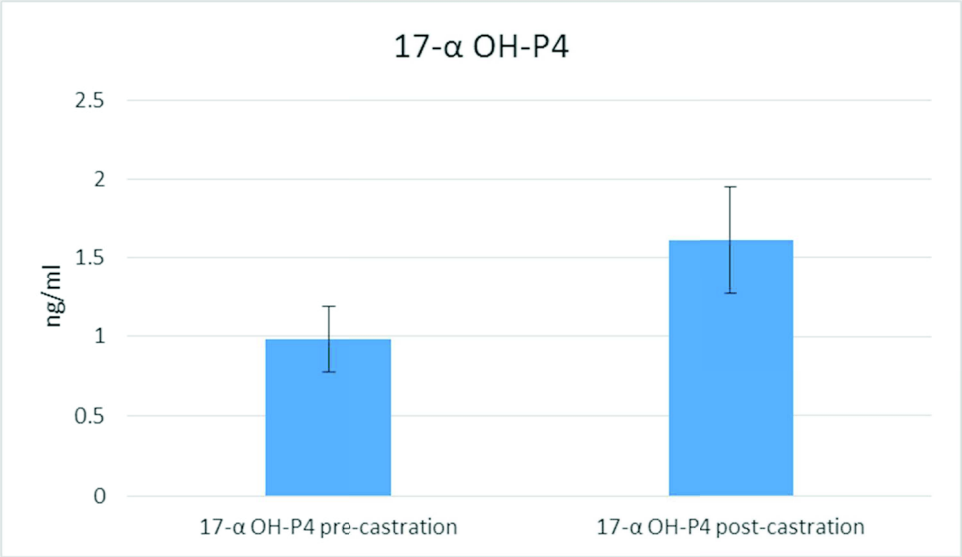


Figure 10: Concentration of 17-α-hydroxyprogesterone

There was a significant difference in 17-α-hydroxyprogesterone values pre and post-castration (Mean= -0.63 ±0.297, T= -2.132, DF=18, P=0.047).

4. Discussion

4.1 Testosterone

Testosterone analysis of the serum blood samples collected prior to and once the orchidectomy was complete demonstrated that testosterone levels in the dogs had decreased significantly post-castration, there was no overlap of the basal values (see figure 7) when comparing testosterone levels in the intact and gonadectomised male. It indicated that the primary source of testosterone in male dogs is from the testicles, thus the decrease in testosterone value was expected.

Multiple studies have been conducted, with the intention to research testosterone levels in animals exposed to various conditions. One study focused on the effects of GnRH stimulation on the pituitary-gonadal axis in male and female dogs before and after a gonadectomy. In this study, testosterone was measured one week before the gonadectomy and 4 months after the orchidectomy was completed, in both situation GnRH was administered after the basal testosterone sample was taken. The basal testosterone values in the castrated dog had decreased significantly from 17.44 nmol/L in intact males to 0.06 nmol/L four months post-castration, supporting the theory that the gonads are responsible for producing the circulating testosterone levels in an intact dog. Although testosterone values had decreased in the gonadectomised dog, it was still detectable in small concentrations at 0.06 nmol/L, indicating that there is an extra-gonadal source of testosterone production in the dog, most likely originating from the zona reticularis of the adrenal cortex (De Gier et al., 2012).

Testosterone values obtained from the measured shelter dog samples, demonstrated that the decline in testosterone levels occurred rather quickly in the animal, see table 6. An additional study regarding the changes in blood testosterone levels in dogs after either a surgical or chemical castration was undertaken, the study highlights the decrease in testosterone levels in the castrated dog using a blood sample obtained one hour after completion of the surgery. The study suggests that there was only a slight decrease in testosterone levels when compared to the control sample obtained six months prior, whilst an additional sample obtained four months after the surgical procedure, displayed testosterone levels had decreased below their proposed cut off point of 1 ng/ml.

Considering the primary source of production in the testes has been removed, the assumption can be made, that testosterone levels in castrated male animals will not return to the pre-castrated intact levels.

4.2 DHT

The significant decrease in DHT values measured post-castration is in correlation with testosterone decline. As DHT is a metabolite converted from testosterone. The high levels circulating DHT in the male dog prior to castration, corresponds to the presence of functional testicular tissue. The measured mean DHT value declined post-castration to 2076.30 pg/ml in our study. The control animal, who had been castrated previously, displayed fluctuations in DHT levels in the blood when measured, ranging from 126.83-109.50 pg/ml. This would suggest that there is an additional source of DHT in the animal, excluding the gonads. A study in mice confirms both the decline in DHT blood levels post-orchidectomy and a relative maintenance of DHT production in the gonadectomised animal (Ando et al., 1986). As previously mentioned, DHT is also produced in the prostate gland, skin and adrenal glands, notably from its precursor testosterone, thus explaining the DHT values measured in our control.

4.3 Oestrogen

Oestrogen values measured showed no significant difference when the values of intact and castrated males were compared (figure 6). An explanation to the absence of a significant decrease in oestrogen values in the gonadectomised males may be that oestrogen is aromatised from androstenedione and testosterone produced in the adrenal cortex and peripheral tissues such as adipose tissue and hair follicles, thus there is a slower decline in oestrogen values observed (Santen et al, 1980).

4.4 Progesterone and Pregnenolone

Like oestrogen, progesterone (figure 2) and pregnenolone (figure 3) levels did not significantly decline in the gonadectomised dog. As previously mentioned in the literature review, both are intermediary metabolites in the adrenal gland cortex involved in the synthesis of mineralocorticoids, glucocorticoids and androgens. Enzymatic reactions control the path of direction for which progesterone and pregnenolone are to follow to produce the steroid hormone of interest. A potential reasoning behind the absence of a significant decline in both intermediary steroid metabolites in the gonadectomised animal may be, that regardless whether testicular tissue is present or not, there is an independent requirement for progesterone for other steroid hormone production, notably aldosterone and cortisol. This reasoning can be supported with ACTH stimulation tests in both intact and neutered male dogs. Both groups displayed an increase in progesterone levels in response to ACTH administration (Frank et al., 2003).

4.5 17-alpha-hydroxyprogesterone

A significant increase in 17-α -hydroxyprogesterone (figure 9) was measured in blood samples collected in the gonadectomised dog. Mean values post castration had increased from 0.98 ng/ml to 1.61 ng/ml. 17- α -hydroxyprogesterone is an intermediary metabolite in the adrenal cortex that is involved in cortisol production. The significant increase in 17-α-hydroxyprogesterone values may be explained by the stress reaction that occurred during the orchidectomy procedure. The surgical stress response is characterized by an increase of

pituitary gland hormone secretion and activation of the sympathetic nervous system in response to a threat that may disrupt the internal homeostatic environment of the animal. The blood cortisol concentrations measured post-castration showed a significant increase in value, 234.16 nmol/L (table 4). Cortisol is a stress-related adrenal hormone, produced during a stress response. The significant increase of cortisol measured post-castration in indicative that a stress response occurred during the orchidectomy. The rise is cortisol may have been influenced by the anaesthesia or the surgery, a study concluded in horses is supportive of this theory (Ayala et al., 2012).

4.6 Cortisol and Corticosterone

Cortisol blood levels measured prior to the orchidectomy were found to be at the higher value range (figure 5). Normal cortisol values in dogs ranges between 13.8-137.9 nmol/L (Klein & Peterson., 2010). Indications for this may be due to the stress of transport to the college, social stress from mixing with strange dogs at the shelter, environment stress as the dogs are being exposed to new surroundings and noises (Villier et al, 1997). The increase in corticosterone values (figure 4), may be indicative of compensatory internal mechanisms to maintain circulatory function and mobilization of energy for organ systems (Sundbom et al., 2011).

5. Conclusion

In male dogs, the steroid hormones, testosterone and DHT have significant roles in both the fetus and as an adult. Presence of the potent male sexual steroids in utero, allows for development of the internal and external male genitalia, whilst in adolescence and at adult age, both DHT and testosterone influences sexual maturity, secondary sexual characteristics and maintains spermatogenesis.

Past studies have indicated, that upon removal of the testicles, the primary source of sexual steroids decreases to much lower concentrations, however it appears that internal mechanisms within the body attempt to compensate for the loss of the primary source of sexual steroids, via adrenal gland production. This may be of significance, considering the additional physiological functions that steroid hormones play important roles in; testosterone inducing vasodilation within the cardiovascular system, estrogen production within the brain if neurodegeneration has occurred.

Blood anaylsis for steroid hormones post-castration, have revealed that the sexual steroids are continuously being produced albeit at much lower concentrations, indicating an extra-gonadal source of production, thus suggesting a larger role in which the sexual steroid hormones play regardless if the animal is intact or castrated.

6. Abstract

The purpose of this study was to measure the short term effects of castration on steroid metabolism in male shelter dogs. Blood samples were obtained from 20 male dogs that were part of an on-going neutering program run at Szent Istvan Univeristy, Faculty of Veterinary Science, Budapest, Hungary. Two blood samples were obtained from the individual dogs prior to and post castration. Serum concentrations were determined in both samples for progesterone, pregnenolone, 17α-hydroxyprogesterone, di-hydrotestosterone (DHT), testosterone, oestrogen, cortisol and corticosterone. Prior to the surgery the intact male dogs had greater concentrations of DHT, testosterone, progesterone, pregnenolone and oestrogen. Blood steroid concentrations measured after the surgery demonstrated a significant increase in cortisol, corticosterone and 17α-hydroxyprogesterone levels, where $P<0.05$. There was a highly significant decrease of testosterone and DHT concentrations measured post-castration, where $P<0.001$. There was no significant changes in serum concentrations of progesterone, pregnenolone and oestrogen measured from the two samples.

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