Emerging roles for vitamin D and prolactin in canine male reproduction

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Abstract

Vitamin D and prolactin, known for their traditional roles in calcium homeostasis and lactation, respectively, are emerging as important factors in human prostatic health and semen quality. Recent research to elucidate the function of these elements in human male reproduction will be briefly reviewed with an emphasis on exploration of new avenues of study in reproduction in the stud dog.

Keywords: Vitamin D, prolactin, dog, reproduction

Introduction

From a veterinary clinical perspective, vitamin D (VD) and prolactin (PRL) are well known for their roles in preventing rickets and in the normal physiology of preparing the mammary gland for lactation and for maternal behaviour in the dam, respectively. However, it appears that multiple physiological and or pathological roles can be attributed to both these factors such as a decreased mortality risk with prostatic cancer\(^1\) and benign prostatic hyperplasia\(^2\) (BPH) in men with adequate VD intake and a role in decreasing semen quality and prostate size when PRL levels are greater than normal.\(^3\)

This brief review of the literature will look at the effects of both these compounds in male reproduction and possible new avenues of research into prostate health and semen quality in the dog.

The physiology of vitamin D

Vitamin D is a fat-soluble vitamin and pro-hormone that exists in two forms; cholecalciferol (VD2) from plant sources and ergocalciferol (VD3) found in animal sources.\(^4\) Unlike humans, carnivores cannot synthesize VD3 through the action of ultraviolet (UV) radiation on the skin and as a consequence, it must be obtained strictly through the diet from sources such as liver or fish oils.\(^4\) Once ingested, VD3 is stored or converted in the liver to 25 hydroxycholecalciferol (25OHVD3), the main circulating form in plasma. Enzymatic conversion to VD3 is dependent on the amount of VD3 ingested, liver function and circulating 25OHVD3 concentrations in plasma.\(^5\) In calcium and phosphorous homeostasis, 25OHVD3 is further converted by 1\(\alpha\)-hydroxylase in the kidney to the biologically active form 1,25 dihydroxycholecalciferol (1,25diOHVD3), otherwise known as calcitriol or 24,25 dihydroxycholecalciferol in the kidney. 1\(\alpha\)-hydroxylase is controlled by a variety of factors such as the plasma concentrations of calcium, phosphorus, parathyroid hormone and VD3 metabolites.\(^5\) However, it is the non-traditional role of VD3; mainly its anti-proliferative effects and its action as a mediator of cell differentiation and apoptosis in multiple tissues such as the prostate and testis that is of greatest interest.\(^6,7\)

The enzyme 1\(\alpha\)-hydroxylase is found in multiple tissues, including the prostate gland in humans and rats. After conversion to calcitriol, this biologically active form binds to the cytosolic vitamin D receptor (VDR), a heterodimer is formed with retinoid X receptor (RXR) in order to pass into the nucleus and bind to VDR response elements (VDRE) to initiate nuclear transcription events and/or to suppress them.\(^7\) With regards to male reproduction the VDR has been identified in human and rat prostate and testes (seminiferous tubules and spermatozoa)\(^9-11\) as well as in prostatic tissue in the dog (Figure).\(^12,13\)
Figure

Vitamin D and the prostate

Recent cancer statistics in the human male population have shown that prostate cancer is the second most diagnosed cancer and has the second highest mortality rate in men next to lung cancer,\textsuperscript{14} while BPH was found to occur in three out of four men in their seventh decade.\textsuperscript{15} Although, the occurrence of prostatic carcinoma is relatively rare in the dog at less than 4% reported by Mukaratirwa,\textsuperscript{16} BPH prevalence is similar to their human counter-parts with an estimated >80\% of dogs having BPH by the age of four years.\textsuperscript{17,18} Early epidemiological studies into risk factors of prostate cancer\textsuperscript{19} found a strong negative association of UV exposure and mortality risk of prostatic carcinoma from which the authors hypothesized a role for VD in the pathogenesis of the disease. Conversely, meta-analysis of 11 relevant studies investigating risk of prostate cancer and VD levels however, showed no association between circulating 25OHVD levels and prostatic cancer risk.\textsuperscript{20} However, the role of VD appears to be more complex than simply looking at circulating serum levels. Certain prostate cancer lines do not express the gene for 1\(\alpha\)-hydroxylase and increase production of 24-hydroxylase to inactivate 25OHVD and early clinical trials in human medicine have shown a synergistic effect among calcitriol, dexamethasone and ketoconazole – a potent inhibitor of 24-hydroxylase – that resulted in a synergistic decrease of prostate specific antigen (PSA) levels in human patients\textsuperscript{21} – a biological marker for prostate disease in men.

In the case of BPH, prostatic cells undergo hyperplasia – not dysplasia – and therefore retain the characteristics of normal prostatic cells. This process is mediated through the action of the androgen dihydrotestosterone (DHT), formed from testosterone (T) by the action of 5\(\alpha\)-reductase within the prostatic epithelium.\textsuperscript{17} Although greatest treatment effects are mediated through the action of 5\(\alpha\)-reductase inhibitors such as finasteride, a multifactorial influence on prostate size exists, of which the VDR has attracted some attention. Both \textit{in vitro} and \textit{in vivo} studies in rats and humans\textsuperscript{22-27} have tested VD analogues such as elocalcitol with affinity for the VDR, yet without the calcemic effects seen with the use of calcitriol. Indeed, it is the toxic effects of VD that limit the dose and frequency of calcitriol in treatment studies.\textsuperscript{21} Elocalcitol, used on human BPH tissue culture \textit{in vitro} was shown to decrease the number of cells via apoptosis in the presence of T, by more than 40\%.\textsuperscript{24} In the same study, a similar
effect was seen in decreasing rat prostate weight in vivo by 30% compared to 40% in the finasteride treated group. These in vivo studies also showed no effect of elocalcitol on T and luteinizing hormone (LH) levels suggesting control of proliferation downstream of androgen action and lack of significant side effects on the pituitary and testes. Adorini et al tested elocalcitol in six beagle dogs prior to clinical trial in humans and observed a decrease in prostate weight over a nine month treatment period that persisted until the end of the two month recovery period. However, statistical significance could not be reached possibly due to the small sample size.

Vitamin D and semen quality

There are few studies in the literature involving VD and its role in male fertility, namely semen quantity and quality characteristics. In rats, it was shown that fertility rate decreased in vitamin D deficient male rats based on pregnancy rates and presence or absence of sperm in the vagina after copulation compared with the VD supplemented controls. Furthermore, reduced testicular and epididymal sperm count, decreased numbers of Leydig cells, degenerative changes in germinal epithelium and lowered glutamyl transpeptidase activity in Sertoli cells was shown in a study by Sood et al and supported by identification of the VDR in rat Sertoli cells. To determine the role of the VDR, Kinuta et al studied VDR null mice and found a dramatic reduction in sperm count and sperm motility compared with controls. Histologically, thinning of the seminiferous epithelium was noted with dilated seminiferous tubules and decreased or infrequent spermatogenesis in the testes of the mice. Interestingly, those VDR null mice that had calcium supplementation with normal serum calcium levels did not show signs of decreased fertility.

In humans the VDR has been identified in the head of sperm cells but is lacking in the neck and tail region. Cholesterol efflux – a priming event in the phosphorylation of proteins leading to human sperm capacitation – was increased in the presence of calcitriol. Also, an increase in phosphorylation of tyrosine and threonine suggests that the VDR has a role in capacitation and survival of sperm. Blomberg-Jensen et al were able to identify the VDR and the enzymes of VD metabolism in the human testis, epididymis, prostate, and seminal vesicles in varying degrees. Currently, canine studies investigating presence or action of VD or the VDR in testes or sperm do not exist.

The physiological role of prolactin

Prolactin is a 23kDa pituitary peptide hormone related to growth hormone (GH) and placental lactogen (PL) and is produced by the lactotroph cells of the anterior pituitary. It is well known for its role in lactogenesis in the female, however, it is also known to have a role in regulation of male reproduction and is involved in reproductive pathologies in men. It exerts its own negative feedback, through the cerebrospinal fluid, on the dopaminergic cells of the hypothalamus (HT). The main effect of PRL is on the interstitial cells of Leydig by up-regulating LH receptors and increasing the affinity of the Leydig cells for LH thereby stimulating release of T. Testosterone, in turn, stimulates Sertoli cells to produce estradiol-17-β (E2) and androgen binding protein (ABP) and through negative feedback on the dopaminergic cells of the HT decreases PRL secretion. The main hormone of up-regulation of PRL secretion is thought to be E2 through a direct effect on lactotroph cells. However, in cases of hyperprolactinemia in men, where greater than physiological levels of E2 are present, it also exerts an inhibitory effect on gonadotropin releasing hormone (GnRH) release from the HT causing decreased secretion of LH and follicle stimulating hormone (FSH) from the pituitary, a response not seen in normal physiological conditions.

Prolactin reference ranges, ultradian and circannual patterns of PRL secretion have recently been published for the male dog, although a difference in type of assay among these studies should be noted. The ranges published by Corrada et al, using homologous enzyme immunometric assay are summarized in the table. Of note, Beagle dogs had, on average, much higher PRL levels than crossbred dogs and German Shepherd dogs sampled in this study. This same group was able to show a circannual variation in PRL levels with an association of higher levels with increased daylight hours (November, December and January compared to May, June, July in the southern hemisphere). Although, Kreeger et al
recorded nadir during the fall months of October and November, taking latitude into account, both studies saw similar patterns with respect to hours of daylight.

Although, mean PRL concentration measured using radioimmunoassay (RIA) did not differ widely between breeds with normospermia; Urhausen et al found a significant difference between these values in Fox Terriers compared with Great Danes, the latter being lower. It was difficult to draw breed specific differences, however, possibly due to the close genetic relationship between the Fox Terriers enrolled in the study. A sharp increase in PRL levels was observed after thyroid stimulating hormone (TSH) injection in this same study. Mean values of PRL remained within the range specified by Corrada et al, yet it is important to note that sampling was only done once prior to TSH stimulation. Therefore normal PRL fluctuation was not accounted for. Although, semen quality parameters are affected in human males, the study by Koivisto et al, determined that semen parameters and libido remained unchanged after induced short-term hyperprolactinemia of three weeks duration.40

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<th>Table. Prolactin values in dogs</th>
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<td>Range (Mean ± SE) (ng/ml)</td>
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<td>Mean Baseline ± SE</td>
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<td>Pulse frequency (peaks/6hr)</td>
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<td>Pulse duration (mins) (Mean ± SE)</td>
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<td>Pulse amplitude (ng/ml) (Mean ± SE)</td>
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**Prolactin and the prostate**

Prolactin and its receptor (PRLR)41,42 have been identified in human and rat prostatic epithelial tissue. Prolactin has been found to be a necessary component for prostatic epithelial growth and survival in culture.34,43 Both *in vivo* and *in vitro* mouse studies showed PRL to affect growth and differentiation of the prostate44,45 and sensitized the prostatic epithelial cells to androgen effects through synergism between T and PRL producing an increase in 5α-reductase activity.46 This resulted in marked increases in the weight of the gland with histologically detectable hyperplastic changes47 under conditions meant to mimic hyperprolactinemia. Prolactin and PRL binding sites were found intracellularly in canine prostatic cells,48 however, the changes noted in the mouse and rat studies were not found in the dog and increases in cell proliferation were seen only in those cultures supplemented with bovine and dog serum alone and not with any steroid hormones or PRL.49,50 The effect of PRL in the dog could be mediated through downregulation of DHT levels. Helmerich et al were able to show that pretreatment with PRL decreased prostatic tissue levels of DHT with subsequent increased T and was significantly different from controls.51 In this same study, treatment with bromocriptine significantly increased prostatic DHT compared with controls.

A regulatory role of PRL was further supported by the study by Robertson et al. In PRLR knock-out mice, the ventral lobe of the prostate was 20% heavier compared to controls. However, the ratio of epithelial cells to stroma within the dorsal lobe was decreased in PRLR -/- mice. They found that castrated PRLR knock-out mice had greater reduction in prostate size than in normal controls suggesting that PRL and T act together on development of the ventral lobe, with a PRL dependent regulating effect under normal physiological conditions of the hormone.52 This difference in size between PRL null and control mice disappeared at one year of age suggesting there is a transient affect of PRL during prostate development.

Conflicting findings on the role of PRL in male prostate physiology are apparent in the literature, however, it appears that this can be attributed to dose-dependent effects and chronicity as seen with other pituitary hormones such as GnRH agonists. The PRL feedback loop appears to take longer for effect when compared with other hormones.53 Further research is needed; especially in the dog, as even less information is available in this species. This absence of information opens a new door to studying the prostate and conditions such as BPH.
**Prolactin and semen quality**

It is well known that conditions of hyperprolactinemia are associated with semen quality and decreased libido in both rodents and men and depending on the severity of the hyperprolactinemia and chronicity of the condition, hypogonadism may be present. The main effects on semen quality include oligozoospermia, asthenozoospermia and teratozoospermia in these species. Many of these defects can be corrected with treatment of dopamine agonists or PRL antagonists although slight elevations in serum PRL seem to have little effect on male reproductive characteristics. In contrast to the female, PRL deficiency appears to have no effect on male fertility as is shown with PRL null mice and rats being able to sire normal litters with no reduction in libido or semen quality. In the dog, Koivisto et al found a significant, if small, increase in mean straight-line velocity in the spermatozoa of those dogs treated with cabergoline.

**Conclusions**

Vitamin D and prolactin have been shown to be involved in reproductive physiology. Ongoing research is helping to better understand these elements and to determine how their effects may be used to develop new treatment strategies for both prostatic and infertility issues. Although, species differences exist between human and canine reproduction, basic reproductive physiological characteristics may warrant further study to elucidate the possible roles of VD and prolactin in the stud dog and promises to be a fascinating area of research in this species.

**References**