

# Canine prostate models in preclinical studies of minimally invasive interventions: part II, benign prostatic hyperplasia models

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**Abstract:** Canine prostate is widely used as animal model in the preclinical evaluation of emerging therapeutic interventions. Spontaneous benign prostatic hyperplasia (BPH) is common in adult intact male dogs with two distinct pathological types: glandular and complex form of prostatic hyperplasia. The complex form of prostatic hyperplasia, usually occurring in older dogs, represents an ideal model because of its unique pathologic feature, including not only glandular hyperplasia but also an increase in prostate stromal components. The limited commercial availability of adult dogs with spontaneous BPH motivates experimentally induced BPH in young dogs. Hormone-induced canine BPH model has been well established with various hormonal treatment regimens and administration approaches. The goal of this review is to provide the veterinary background in spontaneous BPH in dogs, summarize the techniques in hormonal induction of canine BPH, and highlight the pathological and clinical limitations of the canine models that may lead to distinct therapeutic responses compared to clinical trials in humans.

**Keywords:** Animal model; benign prostatic hyperplasia (BPH); dog; hormonal therapy; pathogenesis

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## Introduction

Apart from the chimpanzee and macaque, the dog is the only nonhuman species in which spontaneous benign prostatic hyperplasia (BPH) occurs frequently (1-3). The dog has long been used in the research of BPH of its etiology and pathogenesis, and the experimentally induced BPH canine models were finally established after decades of investigation. Currently various canine prostate models are widely used in the development of new therapies for the management BPH because of the anatomic similarity and the applicability allowing performance of the same therapeutic devices and techniques used in men. Proper selection of dogs with naturally occurring BPH or successful creation hormone-induced BPH models is critical to ensure objective evaluation of a particular technique to be tested. In this part of the review, we provide an overview of the veterinary background of spontaneous

BPH in dogs, highlighting distinct pathological features and clinical characteristics compared to human BPH. The methods in creation of different hormone-induced models are described, and the technical limitations of each model that may potentially lead to disparate responses compared to those in clinical trials are discussed.

## Canine spontaneous BPH

BPH is also an age-related disease in dogs as like in humans. Spontaneous BPH in dogs begins as glandular hyperplasia as early as 2–3 years of age; with time, it may occur in any intact male dog without breed predisposition. It has been documented that BPH is present in almost 100% of adult intact dogs over 7 years old (4,5), and prostate size in affected dogs has been reported 2–6.5 times greater than that of normal dogs of similar body weight (6). In

a pathological study in 41 beagles ranging in age from 1–10 years, DeKlerk *et al.* (7) observed an increase in prostate wet weight with age from a mean of  $14.7 \pm 6.4$  g ( $\pm$  SD) in animals 1–3 years of age to  $23.65 \pm 10.45$  g in those 5–10 years old. BPH was identified in 25% and 88% in groups of 1–3 and 5–10 years of age, respectively. Of note, all prostates in the 41 dogs that weighted more than 18 g had pathological evidence of BPH; whereas all except one that weighted less than 12.8 g were histologically normal (7). Therefore, using this gravimetric criterion, an adult beagle with the prostate larger than 18 g can be used as a spontaneous BPH model for animal experiments.

### ***Pathological features of canine spontaneous BPH***

In spontaneous canine BPH, two principal pathological patterns have been documented. The initial hyperplasia begins as glandular hyperplasia, which is observed in dogs up to 4 years of age. This glandular hyperplasia appears as increase in size of alveoli and in degree of papillary infolding as well as increase in amount of secretory epithelium (7,8). In dogs older than 6 years of age, cystic hyperplasia, namely the complex form of hyperplasia, is usually observed (7,8). The typical pathological features in the complex form include a mixture of glandular hyperplasia together with foci of atrophic or attenuated secretory epithelium, and increase in stroma such as smooth muscle and collagen (7,8). According to DeKlerk's observation, approximately one third of spontaneous BPH in beagles younger than 3 years represented the complex form, which increased up to 50% in beagles of 5–10 years (7). Lowseth *et al.* (9) compared age-related changes in the prostate in beagles and found that all dogs of 6 years and older had evidence of complex form of BPH. In diffuse complex BPH, additional pathological findings may be observed, such as significant periglandular chronic inflammation composed primarily of lymphocytes and large mononuclear cells (7,9,10).

Overall, spontaneous canine BPH is an excellent animal model in studying the development of BPH in humans; however, when the spontaneous model is used in evaluation on therapeutic effects of new minimally invasive interventions, the distinct pathological and clinical features between human and canine BPH have to be noted. In humans, the prostate is anatomically fixed between the symphysis pubis and the rectum; human BPH is a nodular lesion, originating in the transition zone and periurethral region. Thus hyperplastic growth in humans compresses inwardly and impinges on the urethra, producing lower

urinary tract symptoms (LUTS). In dogs, the anatomic location of the prostate is not fixed and varies with increase of the gland size; canine hyperplasia is diffuse throughout the gland rather than nodular growth, developing largely from the peripheral terminal glands (8,11). Thereby canine hyperplastic prostate expands outwardly in all directions, commonly producing rectal obstruction rather than LUTS. Accordingly, the use of urodynamic study or retrograde urethrocytography in dogs with spontaneous BPH doesn't seem to be plausible in testing therapeutic response to interventions (12).

### ***Clinical features of canine spontaneous BPH***

In veterinary practice, common symptoms in BPH-affected dogs include sanguinous preputial/urethral discharge, hematuria or hemospermia, tenesmus, and dysuria, which are usually observed in dogs older than 6 years (13). However, there are typically no clinical signs displayed by dogs with BPH until the hyperplastic gland grows large enough at a late symptomatic stage. In preclinical studies, availability of spontaneous canine BPH model is relatively limited; most are stud dogs obtained from commercial suppliers for laboratory animals and without clinical symptoms. The use of spontaneous BPH in dogs with clinical symptoms above and observation of symptom relief to evaluate therapeutic effects is not practicable.

Prostate specific antigen (PSA) is an important biochemical marker in clinical practice in human prostatic disorders, such as PCa and BPH. PSA is a glycoprotein enzyme produced in the epithelial cells of the prostate. It can be demonstrated in the prostatic tissue using immunohistochemistry and detected in the serum and seminal fluid. Over-production of PSA and/or disruption of the epithelium and alteration in blood-glandular barrier may lead to some diffusion of PSA into the tissue around the epithelium and consequently into blood circulation, causing elevated PSA levels. Roehrborn *et al.* (14) first demonstrated that serum PSA levels were highly correlated with prostate volume in an age-dependent manner in human patients with BPH. They subsequently further validated PSA and prostate volume as good predictors of clinical relief in LUTS and improvement of urinary flow in long-term medical therapy for BPH (15,16). Since then PSA has been worldwide used in the evaluation of clinical progression of BPH and its therapeutic responses to treatment modalities. However, PSA is not detected in canine blood or seminal fluid; alternatively, canine prostatic specific esterase (CPSE)

as its counterpart is identified in canine serum and seminal fluid (17). CPSE is produced by columnar epithelial cells in the prostate under androgenic control and can be inhibited by antiandrogen treatment or surgical castration (18-20). It constitutes more than 90% of the canine seminal proteins (21). Dubé *et al.* (22) compared and analyzed prostatic proteins of canine and human seminal plasma and found that both were related enzymes belonging to the serine-protease class. However, their activity towards synthetic substrates indicated that CPSE was a trypsin-like enzyme, whereas PSA has some chymotrypsin-like activity. In veterinary practice, CPSE has the potential to be a promising diagnostic tool in nonneoplastic canine prostatic disorders. Bell *et al.* (17) reported that serum CPSE concentrations were significantly higher in dogs with BPH (mean concentration, 189.7 ng/mL; n=25) compared with normal intact dogs (mean concentration, 41.8 ng/mL; n=20) and normal castrated dogs (mean concentration, 13.9 ng/mL; n=11). However, the authors also noted that concentrations in dogs with prostatitis and prostatic carcinoma were not significantly different from those of dogs with BPH (17). More recently, a CPSE kit for ELISA test of serum and prostatic plasma sample (Odelis®, Virbac Group) has been commercially available. By the use of Odelis® CPSE test, Lévy *et al.* (23) first reported the CPSE serum levels in 34 dogs with BPH and in 55 normal dogs, and demonstrated that the Odelis® CPSE assay reached the diagnosis rate of BPH with a sensitivity of 97.1% and a specificity of 92.7% based on the threshold of 61 ng/mL (54–67 ng/mL). Although the diagnostic specificity needs further addressing among dogs with various prostate disorders, the CPSE assay offers a valuable tool in animal experiments to evaluate new therapies in the spontaneous BPH canine model.

### Hormone-induced BPH models

BPH is of heterogeneous etiology, involving hormones, aging, growth factors, and stromal-epithelial interactions and neurotransmitters, which may play a role, either singly or in combination, in the hyperplastic process (24). Early investigation of etiology and pathogenesis of BPH have demonstrated that testicular hormones, including testosterone, 5 $\alpha$ -dihydrotestosterone (DHT), androstane diols, and 17 $\beta$ -estradiol (E<sub>2</sub>), play a regulatory role in growth of the prostate. Testosterone is the principal androgen in the circulation. In the blood, 44% of total testosterone is bound to a  $\beta$ -globulin, 54% bound to

albumin, and only 2% of testosterone represents the free form that is able to enter the cells (25). Testosterone serves as prohormone with half-life of approximately 12 min in plasma and is irreversibly converted into two highly biologically active hormones: DHT through 5 $\alpha$ -reduction and E<sub>2</sub> through aromatization. DHT cannot be aromatized so that conversion of testosterone to DHT precludes its conversion to E<sub>2</sub>. In the prostate and other androgen target tissues, testosterone is irreversibly 5 $\alpha$ -reduced to DHT which in turn can undergo reversible conversion to both 3 $\alpha$ - and 3 $\beta$ -androstane diols by 3 $\alpha$ - and 3 $\beta$ -hydroxysteroid oxidoreductases, respectively (26). It was initially assumed that 3 $\alpha$ -androstane diol was a potent androgen because exogenous administration of 3 $\alpha$ -androstane diol could induce prostatic hyperplasia more efficiently than DHT in canine studies (27,28). However, subsequently data indicated that 3 $\alpha$ -androstane diol acts as a better precursor of prostatic DHT than an equal dose of DHT, and 3 $\alpha$ -androstane diol exerts its biological effects only after its oxidation back to DHT (29).

### Walsh and Wilson BPH models

In 1976, Walsh and Wilson first reported successful induction of BPH model with androstane diol in castrated dogs (27). The protocol of Walsh and Wilson's model included young male mongrel dogs (1 to 2 years of age) that were castrated and received hormonal therapy with two effective regimens: 3 $\alpha$ -androstane diol alone and 3 $\alpha$ -androstane diol plus 17 $\beta$ -estradiol. Androgens (testosterone, DHT, or 3 $\alpha$ -androstane diol) and 17 $\beta$ -estradiol were prepared by dissolving 25 mg of androgens and 0.25 mg of 17 $\beta$ -estradiol in 1 mL triolein, respectively. The steroids were injected intramuscularly three times weekly and duration of the treatment lasted for 12 months. In their study, approximately half the dogs receiving 3 $\alpha$ -androstane diol alone and all dogs receiving 3 $\alpha$ -androstane diol plus 17 $\beta$ -estradiol fulfilled the weight and histological criteria for BPH in the dog. The most significant increase in prostate weight occurred within the first 6-month in the study. Compared with 3 $\alpha$ -androstane diol alone, combination of 3 $\alpha$ -androstane diol and 17 $\beta$ -estradiol induced the prostate growth more remarkably, supporting the enhanced or synergistic effect of 17 $\beta$ -estradiol in androgen induction of BPH. However, 17 $\beta$ -estradiol alone, testosterone plus 17 $\beta$ -estradiol, or DHT plus 17 $\beta$ -estradiol failed to induce significant weight change (27). This model confirmed the essential role of androgen in the pathogenesis of BPH in dogs, but also left an open question of interest

why 3 $\alpha$ -androstane-20-one promotes prostatic growth more efficiently than DHT. Subsequently, Moore *et al.* (29) well addressed the mechanism of androgens in inducing prostrate growth by measuring the tissue content of testosterone, DHT, and 3 $\alpha$ -androstane-20-one in the prostate of castrated dogs treated with various hormonal regimens. All animals were mongrel dogs weighing 14–28 kg (average weight: 21.2 kg). The authors successfully reproduced Walsh and Wilson's model in seven dogs with 3 $\alpha$ -androstane-20-one and five dogs with 3 $\alpha$ -androstane-20-one plus 17 $\beta$ -estradiol. By contrast, other groups of dogs treated with equivalent doses of testosterone, DHT, and DHT plus 17 $\beta$ -estradiol, respectively, didn't show evidence of BPH. The results indicated that pathologic growth of the prostate correlated better with intraprostatic concentration of dihydrotestosterone plus testosterone than with that of 3 $\alpha$ -androstane-20-one, even in animals with induced BPH by the administration of 3 $\alpha$ -androstane-20-one. For example, in dogs that received injections of 3 $\alpha$ -androstane-20-one plus 17 $\beta$ -estradiol, the final mean prostate weight increased to 43.5 $\pm$ 9.6 g with tissue content of DHT of 4.40 $\pm$ 0.52 ng/g. This was in sharp contrast to the dogs receiving injections of DHT plus 17 $\beta$ -estradiol, with a final mean prostate weight of 6.5 $\pm$ 1.4 g and tissue content of DHT of 2.70 $\pm$ 0.89 ng/g. On the other hand, the intraprostatic concentration of 3 $\alpha$ -androstane-20-one in the groups treated with androgens plus 17 $\beta$ -estradiol was similar, despite the fact that the weight of the prostates in the group treated with 3 $\alpha$ -androstane-20-one plus 17 $\beta$ -estradiol was on average six fold greater than those in animals treated with DHT plus 17 $\beta$ -estradiol. Furthermore, when castrated dogs were treated with extra-large amounts (375 mg/wk) of DHT or 3 $\alpha$ -androstane-20-one for 12 weeks, the authors observed that DHT was as effective in promoting prostate growth as 3 $\alpha$ -androstane-20-one (22.4 $\pm$ 1.8 *vs.* 21.5 $\pm$ 3.2 g), and the concentration of DHT in the prostate was similar in the two groups (19.4 $\pm$ 4.6 *vs.* 16.8 $\pm$ 2.3 ng/g) (29). This study strongly supports that DHT rather than 3 $\alpha$ -androstane-20-one is the principal intracellular mediator in the development of BPH. Therefore, 3 $\alpha$ -androstane-20-one exerts its remarkable growth-promoting effects on the dog prostate not because of a unique property of the molecule but rather because of some feature of its metabolism that causes it to be an effective precursor of intracellular DHT (26,27). Also, it was speculated that DHT might be cleared more rapidly from plasma and tissues so that the steady-state concentration after its administration would be low and failed to reach the threshold levels in stimulating the prostate growth in the treated dogs (29).

It is worth noting that Walsh and Wilson's model was established when investigating the pathologic effects of exogenous androgens in the induction of the development of BPH, thereby the experimental animals were castrated and then received hormonal therapy. However, in protocols of the conventional model, the duration of castration before commencing the treatment was not controlled, namely the timing to start hormonal therapy (immediately, 2 weeks or 1 month after castration) was not defined; this may potentially affect the outcomes of the treatment and lead to mixed findings in the development of BPH models. Jacobi *et al.* (30) have demonstrated that castration causes a profound decrease in the enzymes that interconvert DHT and 3 $\alpha$ -androstane-20-one and the duration of prior castration has a substantial influence on androgenic responses in hormonal therapy in dogs. The other flaw of the conventional Walsh and Wilson's model is the use of mongrel dogs with a wide range of body weight, in which the defined dosage of 25 mg of 3 $\alpha$ -androstane-20-one and 0.25 mg of 17 $\beta$ -estradiol was injected. The varied doses based on significant differences in body weight may substantially affect the response of the prostate. Therefore, Walsh and Wilson's model needs to be further refined.

#### *DeKlerk BPH models*

In 1979, DeKlerk *et al.* (7) reported their extensive studies on both spontaneous and experimentally induced canine prostatic hyperplasia in purebred beagles, which well addressed the technical issues of hormonal induction of prostatic hyperplasia and standardized the experimentally induced canine BPH models. In DeKlerk's studies, treatment regimens were similar to that used in the Walsh and Wilson's model (27), namely 25 mg of androgens (testosterone, DHT, or 3 $\alpha$ -androstane-20-one) in 1 mL of triolein alone or 25 mg of androgens plus 17 $\beta$ -estradiol (0.25 mg in 1 mL of triolein) were administered by deep intramuscular injection. The injection sites were rotated between the upper and lower, left and right hind leg, three times weekly (on Monday, Wednesday, and Friday mornings). In the tests in castrated dogs, hormonal therapy lasted 4 months, which started after 1-month recovery from castration that allowed the complete involution of the prostate in castrated dogs. The protocols by DeKlerk standardize the BPH model under the controlled condition in commence of hormonal therapy and make the model more reproducible. Another modification in DeKlerk's studies

is the use of purebred beagles instead of mongrel dogs as in the Walsh and Wilson's model. Beagles of 1.5–3 years of age have a relatively small body size and a narrower range of body weights compared to mongrel dogs used in previous studies. For example, in DeKlerk's studies the mean body weight before treatment was  $10.9 \pm 1.2$  kg, whereas in a previous study (29) the mongrel dogs had an average weight of 21.2 kg with a range of 14–28 kg, indicating the steroids doses per kg of body weight in beagles doubled when compared with previous studies in mongrel dogs. This may explain the reason for the positive findings in the induction of BPH with DHT plus  $17\beta$ -estradiol in DeKlerk's study and negative in the Walsh and Wilson's model (26,27). By the use of hormone regimen of  $3\alpha$ -androstane- $20\alpha$ -diol (25 mg) plus  $17\beta$ -estradiol (0.25 mg), 3 times/week, we have successfully reproduced DeKlerk's models in eight young male beagles (weight, 8.8–14.7 kg), in which the mean prostate volume increased from  $12.8 \pm 1.8$  mL before surgical castration to  $31.43 \pm 6.47$  mL after 3-month hormonal therapy (31).

In addition, a remarkable contribution in DeKlerk's studies is the development of a hormonal inductive BPH model in intact beagles, namely DeKlerk's intact BPH model. Success in induction of BPH in young beagles with intact testes was found in those treated with either DHT or  $5\alpha$ -androstane- $20\alpha$ -diol, alone, or with each of the two steroids in combination with  $17\beta$ -estradiol. In contrast, the induction of BPH in young castrated beagles, in which the gland had been allowed to involute for 1 month, required the administration of both  $17\beta$ -estradiol and either  $5\alpha$ -androstane- $20\alpha$ -diol or DHT (7).

The difference in pathological background between the induction of DeKlerk's castrated and intact models involves endogenous testicular hormones and involution of the prostate secondary to castration. In the intact model, the endogenous testosterone, DHT and  $3\alpha$ -androstane- $20\alpha$ -diol are negligible in the process of BPH induction with the administration of pharmacological doses of androgens (75 mg/week) when compared with the castrated model. However, the testicular estradiol may play a role in intact dogs treated with androgens alone. In men, approximately 50% of the active estradiol can be produced as a result of aromatization of adrenal androstenedione to estrone that is subsequently reduced to estradiol in peripheral tissues; the remaining testicular estradiol originates either from direct secretion of Leydig cells or from aromatization of the circulating testosterone. If it were the case in dogs, it might mean the total levels of endogenous estradiol in intact dogs

may be doubled compared to castrated dogs. Considering the enhanced or synergistic effect of estradiol in androgens induction of canine prostatic hyperplasia, this may partially explain why in DeKlerk's studies the administration of DHT or  $3\alpha$ -androstane- $20\alpha$ -diol successfully induced BPH in intact animals but failed in castrated dogs (7). Castration triggers rapid involution of the prostate due to apoptosis and atrophy, thus substantially diminishing intraprostatic enzymes activities, such as those of  $5\alpha$ -reductase and  $3\alpha$ -hydroxysteroid dehydrogenase (30). Accordingly, it is reasonable to hypothesize that  $5\alpha$ -reductase activity in the prostate might greatly decrease 1 month after castration in dogs. This may explain why the administration of DHT plus  $17\beta$ -estradiol rather than testosterone plus  $17\beta$ -estradiol induced BPH in the castrated beagles (7). The decreased  $5\alpha$ -reductase activity in castrated dogs is further supported by DeKlerk's observation that intact beagles receiving treatment with testosterone alone showed an increase in overall prostate weights by 199%, and 40% of them had pathological evidence of prostatic hyperplasia; whereas prostate weight increased by 120% in castrated dogs, none of which had induced prostatic hyperplasia (7). Taken together, the treatment regimens of both  $5\alpha$ -androstane- $20\alpha$ -diol and DHT in combination of  $17\beta$ -estradiol are reliable in the induction of canine BPH; the former seemed more effective not only in the significantly increased prostate sizes but also with more incidence in glandular hyperplasia evaluated in the pathological study. DeKlerk's intact and castrated models are comparable in induction rate of BPH and its effectivity. However, DeKlerk's intact models are advantageous over castrated models when used to test various minimally invasive therapeutic therapies. The procedures in creation of hormone inductive BPH models is simpler in intact beagles without surgical castration, shortening the overall induction period without prostatic involution after castration and making the intact model more economic in animal experiments. More importantly, DeKlerk's intact model preserves testes and allows the evaluation on sexual functions, particularly in the test of erectile and ejaculation function, and semen analysis including semen composition, sperm count, morphology, motility, and vigor (32).

It is important to note that pathogenesis of BPH in men is a chronic process that lasts years or decades. Hence, it is impossible to expect any animal models based on medical manipulations and observation for weeks or months to recapitulate the all pictures in pathology of BPH in men. Although DeKlerk's model showed the induced

lesions identical in pathology to the glandular hyperplasia that occurs naturally in the aging dog, no complex form of hyperplasia, similar to human stromal predominant hyperplasia, was observed (7,33). The distinct pathological features may dictate disparate responses to a certain therapy and highlights the limitation of the hormone-induced model. Currently, most minimally invasive interventions in the management of BPH and PCa exercise therapeutic effects by directly inducing local coagulative necrosis. In men, during and after the necrosis formation, the local survival fibromuscular stroma takes part in self-repair resulting in fibrosis and scar formation with contraction. When the necrosis lesion is large enough, the necrotic tissue is sloughed irregularly leaving a relatively small cavity at a late stage up to 12 weeks to 6 months (34–36). In contrast, canine diffuse glandular hyperplasia consists of massive epithelial cells secreting a large amount of prostatic fluid into the lumina of enlarged alveoli. After treatment, coagulative necrosis occurs accompanying with early liquefaction and smooth intraprostatic cavity formation, which was observed early at 24 hours to 3 weeks after interventions (37,38). With time the liquefied tissue is absorbed, the cavity shrinks and the prostate decreases in size (39). Gottfried *et al.* (36) conducted a comparative study on transurethral laser ablation of the prostate in dogs and men. After laser treatment with the same fiber system, laser and wavelength and with comparable operative techniques, the mean reduction of prostate volume was 50% at 3 months and 21% at 6 months in the canine and human prostate, respectively (36). In contrast to the canine prostate which showed consistent findings in cavity formation after laser treatment (37), the intraprostatic cavity was only detected in men in a range of 30–48% after thermal ablations (35,36,40). The presence of a cavity after thermal ablation is of clinical implication because of its positive correlation with an improvement in urinary performance and relief of outlet obstruction (40). In our previous studies with PAE in a DeKlerk's model, various sizes of intraprostatic cavities were observed in all beagles 1 month after embolization (31); whereas it rarely occurred and was observed in only one out of eleven patients with BPH after prostatic artery embolization (41). Interestingly, our findings were inconsistent with a similar animal experiment by Brook *et al.* (42). In this study, 12 old male intact beagles with a mean age of  $6.9 \pm 1.4$  years underwent PAE with the same embolic agents of various particle sizes. These old beagles may be considered a spontaneous BPH model with likely complex form of hyperplasia. In a group

of four beagles embolized with 300–500  $\mu\text{m}$  particles, small cavitory necrosis was detected in only one animal 1 month after embolization. This is in sharp contrast to our findings that in all seven beagles of DeKlerk's castrated model showed large- or middle-size cavities 1 month after embolization using the same size of microsphere particles (40). The inconsistent therapeutic responses may be attributed to the distinct pathological features of the animal models. Accordingly, old beagles with spontaneous BPH, particularly those with complex form of BPH, are the model of choice, if available, to be used in the evaluation of therapeutic interventions in preclinical studies.

### *Silastic implants for steroid delivery*

Conventional hormone-induced BPH models need chronic intramuscular administration of steroids 3 times weekly. Alternatively, a novel method of steroid-filled Silastic implants has also been reported in the effective induction of canine BPH models (43–45). Silastic is a silicone rubber of polydimethylsiloxane polymer made by Dow Corning Corporation. The silicone rubber has various striking physical features and biological properties, including flexibility, translucency, high-temperature and chemical resistance, high resilience and elastic memory, and high biocompatibility without foreign body reaction, allergenic or thrombogenic behaviors. It is widely used in medical devices and surgical prostheses. Early observation by Folkman and Long (46) indicated that silicone rubber has the property of absorbing certain dyes, such as rhodamine B, and subsequently giving off these dyes, suggesting silicone rubber might be used as a drug carrier for prolonged medical therapy. A variety of drugs were initially tested and demonstrated the ability of pass through implanted silicone rubber capsules; these included nitrogen mustard, penicillin, vitamin B<sub>12</sub>, digitoxin, tyrosine, triiodothyronine, histamine, and atropine (46,47). Since then, the high permeability of silicone rubber has been recognized, not only allowing the diffusion of various drugs of small molecules, steroids, or even protein, either in the dry powder form or in a solvent, but also the diffusion of gases, for example, oxygen, carbon dioxide, water vapor, ether, nitrous oxide, halothane, and cyclopropane (48–50). In theory, the quantity of the drug filled in the silicone rubber capsule (tubing) determines the duration of the therapeutic activity. It has been demonstrated the drug diffusion rate from the silicon rubber tubes is governed by local temperature. Furthermore, the rate of transfer is directly proportional to surface area of the

silicone rubber capsule, and inversely proportional to the wall thickness of the capsule (46,49).

The use of subcutaneous Silastic implants containing androgen was first tested in dogs by Vincent *et al.* (51). In this study, 7.0 cm long silicone rubber tubes (3.35 mm ID × 4.66 mm OD, polydimethylsiloxane, Dow Corning, Midland, MI) filled with crystalline testosterone and sealed at both ends were implanted subcutaneously in castrated male dogs. Plasma testosterone was measured prior to and after implantation, after castration with capsules *in situ*, and after capsule removal. It was observed that in the castrated dogs implanted with three or five Silastic-testosterone capsules, plasma testosterone had been restored to normal levels similar to those seen in the intact dogs; whereas plasma testosterone levels were lower than baseline values in those castrated dogs receiving only one testosterone capsule. This study demonstrated that the subcutaneous Silastic capsules can be used as an effective delivery approach in chronic administration of testosterone, indicating a potential technique for chronic induction of canine BPH. Success in creation of canine BPH models by means of subcutaneous Silastic-steroid implants were subsequently reported from several laboratories (43-45), among which Juniewicz *et al.* described detailed protocols and showed convincing data supporting dose-dependent hormonal induction of BPH in castrated dogs (43). In this study, the authors prepared steroid-containing Silastic implants using the same Silastic tubing as described above. Each Silastic tubing (8 cm) was totally filled with crystalline 3 $\alpha$ -androstane-20-one or E<sub>2</sub> and sealed with Silastic medical adhesive at both ends of tubing. Adult intact male beagles were anesthetized and small incisions (1 cm in length) were made through the skin on each side of the spine in the thoracic region to create subcutaneous pockets, where one to two capsules were implanted. Surgical castration was immediately performed in those dogs of treated groups. Dogs were randomized into three implants-treated groups with either 5, 10, or 20 Silastic capsules containing 3 $\alpha$ -androstane-20-one and one capsule containing E<sub>2</sub>, respectively. During follow up of hormonal treatment for 99 days, the induction of BPH with histomorphologic evidence was observed in dogs in 10 $\times$ , and 20 $\times$  capsule groups. Furthermore, increase in size and weight of hyperplastic prostates was well correlated with the dosage of steroids treatment in 10 $\times$ , 20 $\times$  capsule and intramuscular injection groups.

Overall, subcutaneous steroid-filled Silastic capsules assures a good constancy of hormonal delivery over a long period of time. Dosage of steroids is readily controlled by

changing the length of Silastic tubing and the number of capsules implanted. Surgical removal of the implants is easy when the therapy is necessary to terminate. The use of the implants has the advantage of being less labor intensive over the multiple intramuscular injections of steroids during long-term hormonal induction of the canine BPH model. However, attention should be paid in surgical implantation of the capsules to avoid local infection that may cause abscess leading to implants failure.

## Conclusions

Canine BPH models offer a valuable platform supporting preclinical investigation on newly emerging therapeutic interventions in regards of the technical feasibility, efficacy, and safety. Spontaneous BPH in old dogs are the preferred model due to the pathological features, intact testes, and the absence of artificial hormonal manipulations. Hormone-induced BPH models in young dogs are well established with induced lesions identical in pathology to the glandular hyperplasia that occurs naturally in the aging dog with intact testes. Thus, hormone-induced BPH can serve as alternatives to the spontaneous BPH model. However, there exist various distinct pathological features of BPH and associated clinical manifestations in dogs and men, leading to distinct therapeutic responses to a particular treatment and limiting the use of canine BPH models in the evaluation of some specific end points in studies. Understanding the difference in pathology and clinical characteristics of BPH between men and dogs is critical to the experimental design and successful performance in preclinical studies.

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## Footnote

*Conflicts of interest:* The authors have no conflict of interest to declare.

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