Clinical Efficacy of the GnRH Agonist (Deslorelin) in Dogs Affected by Benign Prostatic Hyperplasia and Evaluation of Prostatic Blood Flow by Doppler Ultrasound

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Contents

In six German Shepherds dogs, GnRH agonist implants (Deslorelin) were inserted subcutaneously one month after histological confirmation of benign prostatic hyperplasia (BPH). Prostatic volume (PV), characteristics of ejaculate, serum testosterone concentrations and Doppler parameters of prostatic and subcapsular arteries were detected at different time intervals, for 6 month. The prostatic volume showed a significantly reduction starting at day 37. The decrease in sperm concentration, motility and increase in morphological abnormal sperm were observed from day 22 to day 37, when it was no longer possible to obtain the ejaculate. The values of peak systolic velocity and end-diastolic velocity in prostatic and subcapsular arteries showed from day 11 a gradual decrease, significant at day 22 until day 37 and reaching the lowest values at day 52 until the end of observation. The power Doppler pixel intensity of both arteries showed a gradual decrease from day 5 until day 52. In particular, a significant decrease was observed for both arteries from day 11. Testosterone serum concentration decreased to undetectable levels by day 11 until the end of the observations. All these Doppler parameters and testosterone values were positively correlated with the prostatic volume. Furthermore, testosterone values were positively correlated with peak systolic velocity, end diastolic velocity and pixel numbers. The use of implants containing GnRH analogues, even in asymptomatic subjects, is effective for the control of BPH and the application of Doppler exam of prostatic blood flow represent an non-invasive tool for monitoring the response of medical treatment.

Introduction

In dogs, the prostatic gland, in analogy to humans, presents an age-related propensity to develop benign prostatic hyperplasia (BPH) (Leav et al. 2001), characterized by an increase in the number (hyperplasia) and volume (hypertrophy) of prostate cells (Memon 2007). The exact pathogenesis of this condition is not completely understood; however, the dihydrotestosterone has an important role in stimulating enlargement of the canine prostate by enhancing growth of both stromal and glandular components (Russell and Wilson 1994). Moreover, it was reported that estrogens, growth hormone, prolactin and relaxin may contribute to the development of BPH (Wennbo et al. 1997; Grayhack et al. 1998; Kölle et al. 1999; Yoshinaka et al. 2000; Niebauer et al. 2005; Kaplan-Lefko et al. 2008).

Often asymptomatic BPH is frequently diagnosed as an incidental finding during the andrological examination. Presumptive diagnosis is based on the dog's history,

physical examination, prostate fluid examination and prostate imaging (radiology and ultrasonography). A definitive diagnosis is obtained with a biopsy of the gland (Memon 2007). In many cases, treatment is not required especially if dogs are asymptomatic. The goals of treatment are to resolve the clinical signs of the condition, reduce discomfort and restore the dog pain-free quality of life. The surgical removal of the testis represents the first therapeutic choice, but recently, for ethical reasons and animal welfare legislation, restrictions have been imposed on the 'classic approach'. The choice between surgical (orchiectomy) and conservative approach depends mainly on general health of the animal and the necessity to preserve the fertility of the subject.

Different pharmacological agents are reported for the treatment of BPH such as estrogens (Olson 1984), antiandrogens (Tsutsui et al. 2001), 5α-reductase inhibitors, (Iguer-Ouada and Verstegen 1997) and progestagens (Bamberg-Thalen and Linde-Forsberg 1993; England 1997). A recent option for temporary hormonal castration in male dogs relies on the down-regulation of the hypothalamus–pituitary–gonadal axis as a result of a continuous exposure to a GnRH agonist applied via a slow-release subcutaneous implant (Junaidi et al. 2009; Ludwig et al. 2009). In human medicine, many works reported the use of GnRH analogues for BPH treatment (Peters and Walsh 1987; Gabrilove et al. 1989; Gonzalez-Barcena et al.1994), but few works (Goericke-Pesch et al. 2010; Palm and Reichler 2012) reports the clinical efficacy of GnRH analogue implant (azagly-nafarelin) in dogs affected by BPH.

Doppler ultrasonography, in its different application Colour Doppler (CD), pulsed wave spectral Doppler (PW) and power Doppler (PD), is used for the study of pathophysiological modifications of human prostatic glands. (Miyashita et al. 1988; Kojima et al. 1997, 2000; Keener et al. 2000; Tsuru et al. 2002). Kojima et al. (2000) demonstrated that Doppler ultrasound can discriminate between human patients with normal prostate and those with BPH. In dogs, Günzel-Apel et al. (2001) reported the Doppler ultrasound characteristics of prostate vascularization in BPH-affected dogs.

Therefore, this study was carried out to: (i) increase clinical data on the efficacy of GnRH analogue implant (Suprelorin®) in the treatment of BPH affected dogs and (ii) determine the hemodynamic changes of the prostatic blood flow by Doppler analysis during the treatment.

Materials and Methods

Experimental procedure

All subjects underwent to the experimental procedure at day D-30, D0 (day of Suprelorin ® implant), D5, D11, D22 and every 15 days up to 6 months.

In each day of the experimental procedure, all subjects were submitted to physical examination, blood sampling, semen collection and B-mode and Doppler ultrasound scanning of the prostatic gland.

Animals

Six German Shepherd healthy fertile male dogs aged 4–8 years (median 6 years) and weighing 28 to 36 kg (median 32 kg) were examined. All animals were housed in individual box at the same conditions of light, temperature and nutrition. All procedures were carried out according to the Italian legislation on animal care (DL n.116, 27/01/1992).

Semen collection and evaluation

Semen samples were collected by digital manipulation in the presence of a teaser bitch as previously described (Johnston et al. 2001a). The first, second and the third fractions of the ejaculate were collected into three different plastic vials. Immediately after collection, examination of the sperm rich fraction was performed and included volume, pH using a pH indicator paper (Merck, Darmstadt, Germany), total and progressive motility through the observation of 10 μl of fresh semen placed on a glass slide (37.8°C) under a 100× magnification phase-contrast microscope (Nikon Optiphot 2, Tokyo, Japan). Sperm concentration was determined using a Burker counting chamber (Merck, Leuven, Belgium). The percentage of live and dead spermatozoa and the spermatozoa morphology were examined on eosin–nigrosin-stained smears. At least 100 spermatozoa were evaluated per slide.

Ultrasound scanning

The ultrasound machine (Esaote My Lab 30 Gold, Genova, Italy) equipped with a microconvex probe (6.6–8.0 MHz) for Brightness mode (B-Mode) as well as for Doppler ultrasound scanning was used. After shaving of the caudal abdominal region, the dogs were positioned in standing position. All ultrasound examinations were performed by the same operator. The prostatic glands were examined by B-mode and three measurements of length (L) and depth (DL) on longitudinal section and depth (DT) and width (W) on transverse section were taken. The single mean value of each measure was used for determine PV.

Prostatic volume and expected prostatic volume (EPV) was calculated by the following formulas (Atalan et al. 1999):

$$PV(cm^3) = 0.487 \times L \times W \times (DL + DT) : 2 + 6.38;$$

$$EPV(cm^3) = 8.48 + (0.238 \times kg\ body\ weight)$$

The prostate vascularization was visualized by CD and the arterial blood flow was examined outside the gland in a prostatic artery (lateral location) and inside the gland in a subcapsular artery (subcapsular location). The blood flow parameters, determined by PW, were peak systolic velocity (PSV), end-diastolic velocity (EDV), resistive index (RI) and pulsatility index (PI). In particular, the vessels were visualized by CD, then a PW sample volume was positioned in the centre of a vessel and the waveforms with at least three consecutive cardiac cycles were recorded. The values of blood flow parameters were automatically calculated for each waveform. The values obtained on three sweeps were averaged to obtain a single mean value for each measure at each location. The size of the sample volume, which determines Doppler information, was kept constant at 1 mm and all measurements were obtained with an insonation angle <30° and angle correction was applied.

The PD mode was used for colour flow mapping of the prostate in various transverse sections. To minimize variation, standardized presets (depth 5–7 cm, PD gain 58–85%, and PRF 1.0–1.7 kHz) were used throughout the examinations. The PD sample box was positioned to include the entire section of the prostate. Optimal still images displaying the maximum number of colour pixel in lateral and subcapsular locations without flash artefact were recorded. Three different images were taken for each examination in each location. The total number of colour pixels was calculated by a computer-assisted image analysis system using an open source software Imagej (http://rsbweb.nih.gov/ij/).

Prostatic biopsy

In all subject, the transabdominal ultrasound guided prostatic biopsy (Johnston et al. 2001b) was carried out on D-30. The dogs, sedated with 20 μg/kg i.m. of medetomidine hydrochloride (Sedator ®, ATI S.R.L, Ozzano Emilia, Italy), were positioned in dorsal recumbency. The prostate was displayed in the transverse section and the semiautomatic guillottine biopsy needle of 18 gauge (Tsunamii Medical Modena, Italy) was inserted through inguinal abdominal wall in the ventral portions of the right and left prostate lobes. The biopsies were processed for histological examination according to routine methods.

Implantation of GnRH analogue

The Deslorelin implant (Suprelorin ® 4.7 mg Deslorelin; Virbac, Carros, Francia) was inserted subcutaneously in the infrascapular region on D0 after the histological confirmation of BPH.

Blood sampling

All subjects were submitted to blood collection (2 ml) from the cephalic vein in accordance with experimental procedure and also at D1, D3, and D7. Serum was stored at −20°C until assay.

Testosterone assay

Serum testosterone concentrations were determined by a commercial RIA kit (DIAsource ImmunoAssays S.A.

Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium). The intra- and inter-assay coefficients of variation were 3.3% and 4.8%, respectively. Sensitivity was 0.05 ng/ml.

Statistical analysis

All data were analysed by one-way ANOVA followed by Student–Newman–Keuls t-test, except for the data of concentration, total motility, progressive motility and abnormalities of semen that were analysed by the non-parametric Kruskal–Wallis test followed by Mann–Whitney test. $p < 0.05$ were considered statistically significant. Correlations between were determined by the Pearson's coefficient.

Results

All subjects were asymptomatic and the data of clinical and andrological examinations were in the normal range reported for this specie (Johnston et al. 2001b) except for prostatic gland volume. The use of Deslorelin implants did not cause any local or systemic side effects for the entire duration of the observations. In all dogs, the prostate histopathological exam showed the presence of hyperplastic glandular tissue with an increase in secretory epithelium with the normal architecture of the organ preserved. The glandular structures were lined by monostratified and polarized epithelium, with moderate anisocytosis and anisokaryosis. This findings confirmed in all subjects the presence of BPH.

Each individual ultrasonographic exam lasted approximately 20 min to collect all parameters. In all animals, before the hormonal treatment, the form and capsular margins of prostatic gland were regular and the parenchymal echogenicity was homogeneous with a medium to fine texture. In all dogs, the PV before implantation was higher as compared to the EPV (Table 1). The PV showed a gradual decrease significantly ($p < 0.01$) to the D37 and reaching the lowest values at D52, maintaining these dimension for the remaining period of observation (Fig. 1). The semen characteristics were normal until D11(Table 2). Thereafter from D22 the volume, total and progressive motility decreased significantly ($p < 0.01$), while the percentage of morphological abnormalities significantly increased ($p < 0.01$) as reported in Table 2. After D35, the semen could no longer be obtained.

Throughout the observation period, the prostatic artery waveforms recorded by PW were continuous and showed a typical pattern of the high-resistance

Fig. 1. Mean prostatic volume in dogs with benign prostatic hyperplasia following treatment with Deslorelin implants. Prostatic volume (cm^3) evaluated according to Atalan et al. (1999) using prostatic dimensions obtained by B-mode ultrasound. The black arrow indicates the day of implantation (day 0). The dotted line indicates the mean expected prostatic volume of the six dogs. Values are means ± SEM. *$p < 0.01$ vs day 0

vessels characterized by a rapid systolic peak followed by rapid telediastolic decrease with a relative low EDV. The waveforms registered in subcapsular artery were continuous with a characteristic pattern of the low-resistance vessel with a rapid systolic flow followed by long diastolic decrement with a relatively high telediastolic velocity. The values of PSV and EDV in prostatic and subcapsular arteries showed from D11 a gradual decrease, significant at D22 ($p < 0.01$ except for EDV in subcapsular location $p < 0.05$) until D37 ($p < 0.01$) and reaching the lowest values at D52 until the end of observation ($p < 0.01$) (Figs 2 and 3). The values of RI, of both arteries, did not show any modifications (Figs 2 and 3). The trend of PI was similar to RI therefore only RI trend was reported.

The numbers of PD pixel of prostatic and subcapsular arteries showed a gradual decrease from D5 until D52. In particular, a significant decrease ($p < 0.05$ for prostatic artery and $p < 0.01$ for subcapsular artery) was observed for both arteries from D11 (Fig. 4). During the three days after Deslorelin implant, the serum testosterone concentration increased ($p < 0.01$) from basal values of 1.35 ± 0.21 ng/ml to 2.26 ± 0.29 ng/ml and then decreased ($p < 0.01$) to undetectable levels at D11 until the end of the observation (Fig. 5).

The value of PSV, EDV, pixel number were positively correlated ($p < 0.001$) with testosterone concentration; moreover, all these values were positively correlated ($p < 0.001$) with the prostate volume and the coefficients of correlation were reported in Table 3.

Discussion

As far as we know is the first work that report the hemodynamic change of prostatic blood flow in BPH-affected dogs treated with GnRH analogues implants.

GnRH analogues, agonists and antagonists, have been originated from the native GnRH molecule by substitution of different amino acids to increase both the

Table 1. Prostatic volume (PV) and expected prostatic volume (EPV) in dogs with benign prostatic hyperplasia. The volumes (cm^3) were calculated according to Atalan et al. (1999), using prostatic dimensions obtained by B-mode ultrasound one month before treatment

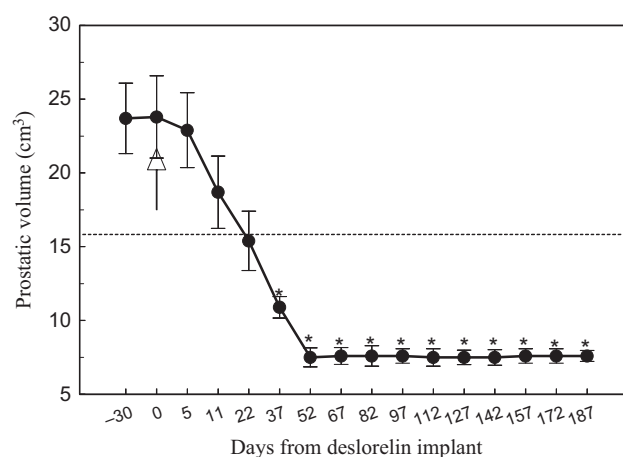| Dog	kg	PV (cm^3)	EPV (cm^3)
1	33.4	26.9	16.4
2	31.4	20.8	15.9
3	32.7	23.6	16.3
4	28.6	17.9	15.3
5	29.8	19.5	15.6
6	35.8	33.7	17.0

Table 2. Qualitative and quantitative parameters of semen samples obtained from six dogs with benign prostatic hyperplasia before and after treatment (day 0) with Deslorelin implant

Day	Semen volume (ml)		Total motility (%)		Progressive motility (%)		Abnormalities (%)		Concentration ($\times 10^6$)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
−30	2.5	0.2[a]	86.2	3.4[a]	77.5	2.6[a]	8.2	1.0[a]	433.4	47.6[a]
0	3.0	0.3[a]	86.3	3.1[a]	77.2	4.0[a]	8.3	1.4[a]	513.7	40.3[a]
5	3.4	0.5[a]	87.7	3.0[a]	79.3	3.3[a]	8.7	0.7[a]	530.6	41.4[a]
11	3.3	0.4[a]	89.2	3.3[a]	81.7	2.2[a]	10.8	1.6[b]	510.0	27.4[a]
22	1.7	0.3[b]	70.2	2.3[b]	61.3	2.6[b]	30.4	3.1[c]	406.3	22.3[a]
35	0.4	0.1[c]	7.1	1.0[c]	8.2	1.3[c]	86.1	3.2[d]	90.2	0.3[b]

Values are means ± SEM. (n = 6). Different letters indicate a significantly different value ($p < 0.01$).

biological activity and the duration of their effects (Gobello 2007). An initial disadvantage for the use of these substances was represented by the numerous administrations required or by the use of minipumps inserted under the skin. A significant step forward was the introduction of slow-release GnRH agonists administered by intramuscular or subcutaneous injection every 3, 6 or 12 months, depending on their formulation (Weckerman and Harzmann 2004). In human medicine, several reports describe the efficacy GnRH analogues for the treatment of BPH (Gabrilove et al. 1989; Geller 1989; Matzkin et al. 1991; Eri et al. 1996), but only one work reports its use in dogs suffering from this condition (Goericke-Pesch et al. 2010).

Deslorelin is an agonist of the hypothalamic GnRH. In our investigation, carried out in asymptomatic dogs with BPH, Deslorelin caused a progressive reduction in the prostate volume as detected by ultrasound scanning. Compared with previous studies involving normal (Ponglowhapan et al. 2002; Ludwig et al. 2009) and BPH-affected dogs (Goericke-Pesch et al. 2010), we observed a more marked (D37 vs D56, D35 and D56) reduction in prostate volume in a shorter period. These differences could be due to the different analogues employed (Goericke-Pesch et al. 2010) or to their use in dogs without BPH (Ponglowhapan et al. 2002; Ludwig et al. 2009).

The development and function of the prostate gland are dependent on androgens. In fact, androgenic steroids regulate prostate size by repressing cells apoptosis and stimulating cell proliferation (Kyprianou and Isaacs 1988; Colombel and Buttyan 1995). The reduction in serum testosterone concentration in this work was positively correlated with prostatic volume, and during the treatment, its levels showed a significant decrease, as reported in previous works (Junaidi et al. 2009; Ludwig et al. 2009; Goericke-Pesch et al. 2010), reaching not detectable value at D11.

The decrease in sperm concentration, motility and increased in abnormal sperm were observed from D22 to D37 when it was no longer possible to obtain the ejaculate in agreement with Junaidi et al. (2009). On the other hand, Ludwig et al. (2009) reported that the ejaculates could no longer be obtained after 3 weeks. This difference is likely due to the employment of different doses and GnRH analogue. The modifications of sperm characteristics and prostatic volume are probably due to the down-regulation of the GnRH

receptors with a consequent marked reduction in the secretion of LH and FSH (Vickery 1985; Vickery et al. 1989; Herbst 2003) and subsequent block of the hypothalamic–pituitary–testicular axis. In fact, the decrease in serum testosterone levels observed in our study, clearly demonstrate a down-regulation of the hypothalamic–pituitary–testicular axis.

In human medicine, the progress in colour Doppler imaging techniques has provided a new potential tool to improve the diagnosis of BPH through the detection of prostatic vascularity by PW and PD (Miyashita et al. 1988; Kojima et al. 1997, 2000; Keener et al. 2000; Tsuru et al. 2002). Kojima et al. (2000) demonstrate that Doppler ultrasound could discriminate between human patients with normal prostate and those with BPH. In our work, the values of PSV and EDV in prostatic and subcapsulary arteries were similar to those previously reported in BPH-affected dogs (Günzel-Apel et al. 2001) and after GnRH implant a significant reduction in both velocity are detected. This significant decrease in PSV and EDV occurred in proportional manner and this is evidenced by the fact that RI did not showed significant modifications. As this index measures peripheral resistance, it is possible to assume that the decrease in blood velocity is caused by factors other than this.

The decrement of blood flow precedes the involution of the prostate in the rat after castration. Androgen action of the prostate might involve primary regulation of prostate blood flow and prostate vascular cell vitality (Lekas et al. 1997; Shabsigh et al. 1998). The evidence that the prostatic vascular system is a primary target of androgen action and other evidence suggesting that the regression of the prostate parenchyma occurs secondarily to the regression of the prostate vascular system through cell death mediated by tissue ischaemia/hypoxia (Buttyan et al. 2000). This could be supported by the fact that in our work the decrease in EDV and PSV and than in the prostate blood flow supply is closely related to the decrease in serum testosterone concentration.

Further technical development in ultrasound has lead to the modern technique of Power Doppler, which exploit the total power in the Doppler signal to produce colour coded real-time images of blood flow. Power Doppler is a technique that differs from conventional Doppler in the way the Doppler signals are processed; instead of estimating mean frequency and variance through autocorrelation, the integral of the power spectrum is estimated and colour coded. Therefore, the
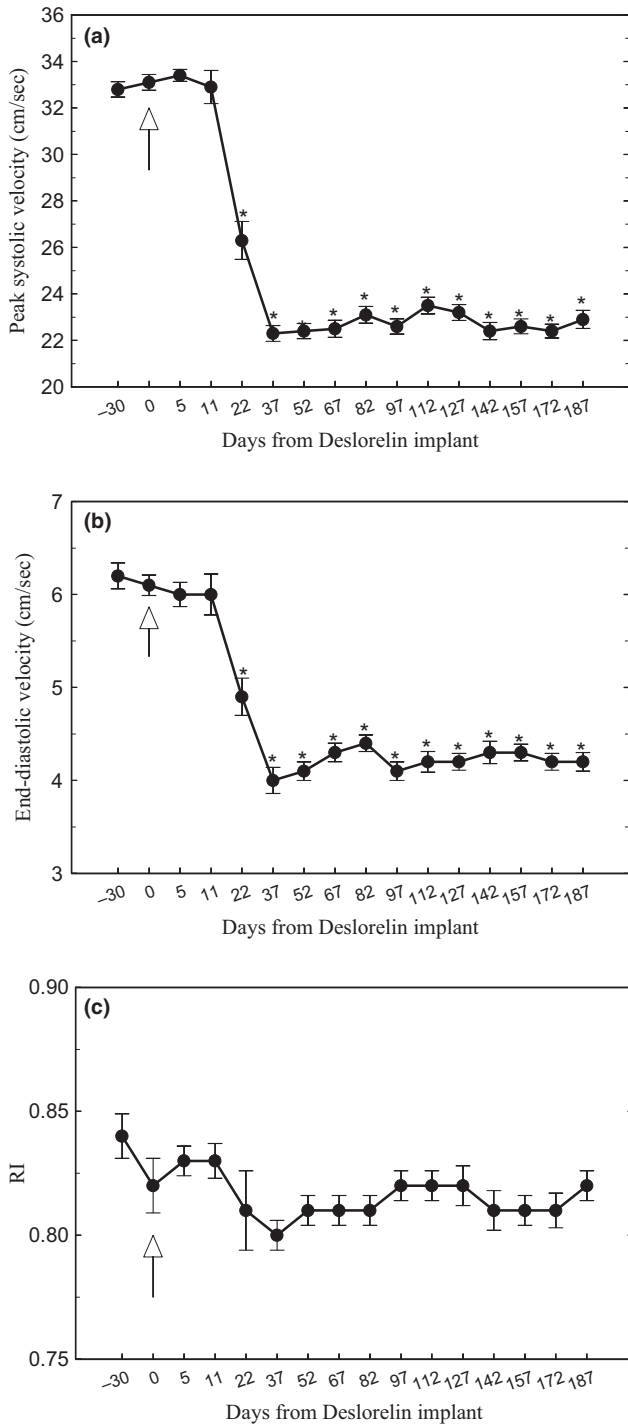
Fig. 2. Hemodynamic parameters of the prostate artery in dogs with benign prostatic hyperplasia (BPH) following treatment with Deslorelin implants. In panel (a) peak systolic velocity (PSV, cm/s), in panel (b) end-diastolic velocity (EDV, cm/s), and in panel (c) resistive index (RI) derived from the prostatic artery in six dogs with BPH between day −30 to day 187 after treatment with deslorelin implant (day 0, arrow). Values are means ± SEM. *p < 0.01 vs day 0
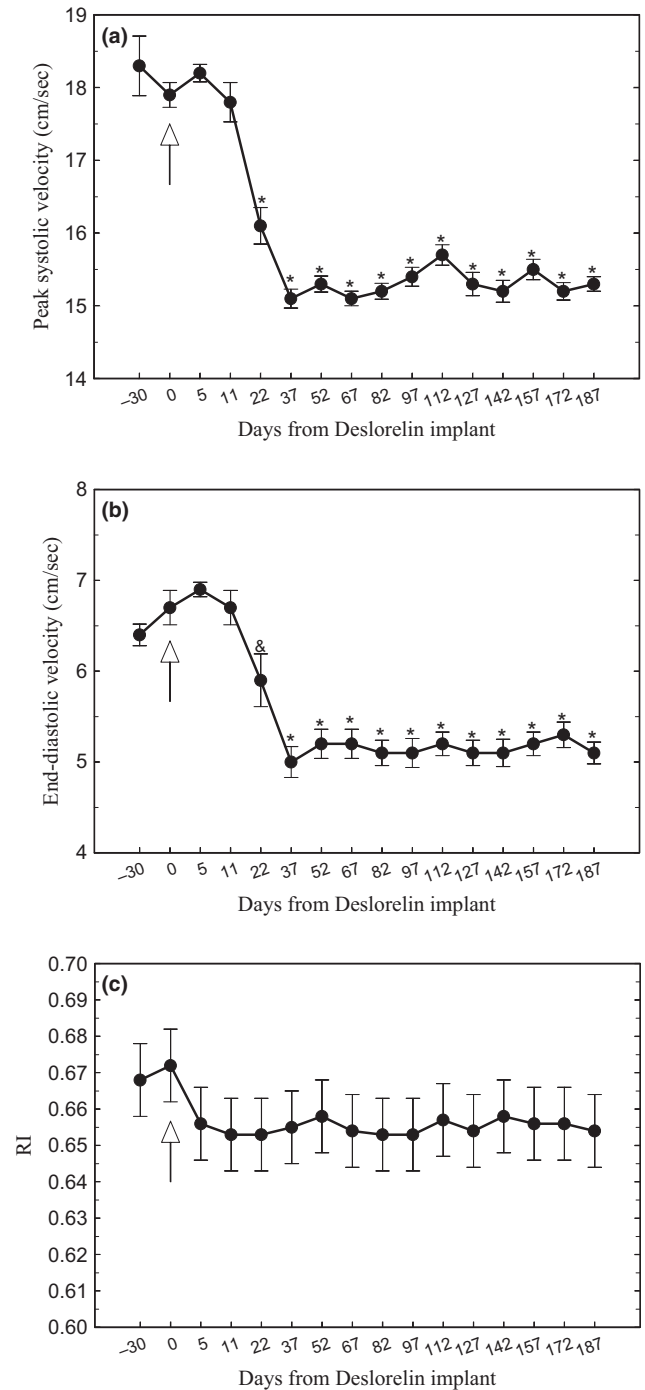
Fig. 3. Hemodynamic parameters of the subcapsular artery in dogs with benign prostatic hyperplasia (BPH) following treatment with Deslorelin implants. In panel (a) peak systolic velocity (PSV, cm/s), in panel (b) end-diastolic velocity (EDV, cm/s), and in panel (c) resistive index (RI) derived from the subcapsular artery of the prostate in six dogs with BPH between day −30 to day 187 after treatment with deslorelin implant (day 0, arrow). Values are means ± SEM. &p < 0.05 and *p < 0.01 vs day 0

total power of the Doppler signal from blood is independent of blood flow velocity and Doppler angle and then is more sensitive to detect blood flow than CD (Kojima et al. 1997). In present work, the pixel number (expression of vascularization detected by means of PD) showed the same trend as PSV and EDV. This further confirms the previous observations that the reduction in

testosterone concentration, by acting on prostate vascularization, reduces the blood flow supply as detected in our work also by PD.

The treatment of BPH includes surgical or pharmacological protocols. In the presence of older males, which can have much higher incidence of surgical complications, orchiectomy is generally not indicated.
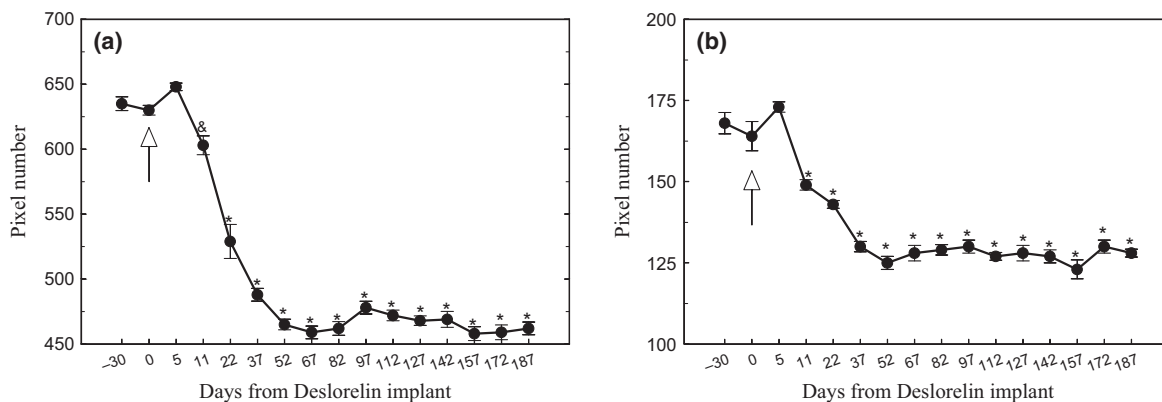
Fig. 4. Pixel numbers in the prostatic (a) and subcasular (b) arteries of dogs with benign prostatic hyperplasia (BPH) following treatment with Deslorelin implants. Power Doppler from prostatic artery (a) and subcapsular artery (b) in six dogs with BPH between day −30 and day 187 after treatment with Deslorelin implant (day 0, arrow). Values are means ± SEM. $^{&}p < 0.05$ and $^{*}p < 0.01$ vs day 0
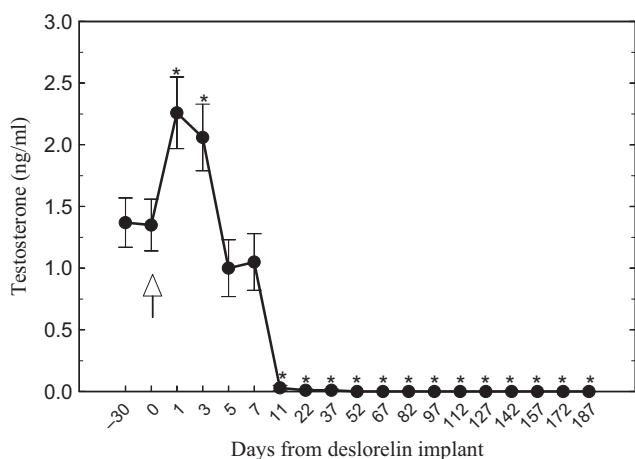

Fig. 5. Testosterone concentrations in dogs with benign prostatic hyperplasia (BPH) following treatment with Deslorelin implants. Testosterone concentrations (ng/ml) in serum samples collected between day −1 and day 187 after treatment with Deslorelin (arrow, day 0) from six dogs with BPH. Values are means ± SEM. $^{*}p < 0.01$ vs day 0

Table 3. Pearson's correlation coefficients among testosterone plasma level, prostate volume and blood flow parameters of marginal and subcapsular vessels obtained from six dogs with benign prostatic hyperplasia after treatment with Deslorelin implant

	Prostate volume	Testosterone
Testosterone	0.863	
Prostatic artery		
PSV	0.998	0.847
EDV	0.978	0.828
PD	0.989	0.843
Subcapsular artery		
PSV	0.985	0.819
EDV	0.960	0.758
PD	0.962	0.889

PSV, peak systolic velocity; EDV, end-diastolic velocity; PD, power Doppler. n = 96; all correlations p < 0.001.

In addition, the choice of conservative approaches vs surgical castration is also influenced by the necessity of preserving the fertility of the subject. In fact, the GnRH agonist treatments cause a temporary and reversible suppression of testicular function (Vickery 1985; Cavitte et al. 1988; Vickery et al. 1989; Trigg et al. 2001; Riesenbeck et al. 2002; Ludwig et al. 2009) also in the presence of testicular fibrous tissue as reported by Ludwig et al. 2009. The success of the pharmacological approach relies not only on the availability of implants with potent long-lasting GnRH agonist but also on the diagnosis of BPH before the appearance of clinical signs. In fact, symptomatic BHP may be present cysts or prostate abscesses that may require surgery.

The positive correlation found between prostate volume and some Doppler parameters suggests that this ultrasound technique can represent a valuable tool for monitoring the efficacy of hormonal therapies. In conclusion, our present work confirms that the administration of Suprelorin is clinically useful for the control of BPH in asymptomatic dogs because prevent progression of prostatic disease and that the monitoring of prostatic blood flow represents a valid and non-invasive tool for the clinical evaluation of BPH and its follow-up during treatment.

Acknowledgement

Conflict of interest

None of the authors have any conflict of interest to declare.

Author contribution

A Polisca the research design and to drafting the paper R Orlandi and A Troisi performed the experimental procedures G Brecchia, M Zerani and C Boiti performed the statistical analysis and hormonal assay R Zelli revising it critically.

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