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Complete List of Authors:	Alonge, Salvatore; Ambulatorio Veterinario "Il Melograno"; Universita degli Studi di Bari Aldo Moro, Department of Veterinary Medicine (DiMeV), Sezione di Chirurgia e Ostetricia Melandri, Monica; Ambulatorio Veterinario "Il Melograno" Leoci, Raffaella; Universita degli Studi di Bari Aldo Moro, Department of Veterinary Medicine (DiMeV), Sezione di Chirurgia e Ostetricia Lacalandra, Giovanni; Universita degli Studi di Bari Aldo Moro, Department of Veterinary Medicine (DiMeV), Sezione di Chirurgia e Ostetricia Aiudi, Giulio ; Universita degli Studi di Bari Aldo Moro, Department of Veterinary Medicine (DiMeV), Sezione di Chirurgia e Ostetricia
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To the Editor of
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Valenzano (BA), May 7th, 2018

Dear Editor,

please find enclosed the manuscript:

"Ejaculation effect on blood testosterone and prostatic Pulsed-Wave Doppler ultrasound in dogs"

by Salvatore ALONGE, Monica MELANDRI, Raffaella LEOCI, Giovanni Michele LACALANDRA, and Giulio AIUDI

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The manuscript has been approved by all co-authors and it is has not been published elsewhere.

Correspondence regarding the paper should be directed to the following address:

Dr. Salvatore ALONGE
Department of Veterinary Medicine (DiMeV)
Sezione di Chirurgia e Ostetricia
University of Bari Aldo Moro, Italy.
Str. Prov. Per Casamassima Km 3
70010, Valenzano, BA, Italy.
Phone +39 392,8058524
e-mail drsalvatorealonge@gmail.com

Thank you for your attention.

Yours sincerely Salvatore Alonge

For Peer Review

EJACULATION EFFECT ON BLOOD TESTOSTERONE AND PROSTATIC PULSED-WAVE DOPPLER ULTRASOUND IN DOGS

Salvatore ALONGE[1,2], Monica MELANDRI[1], Raffaella LEOCI[2], Giovanni Michele LACALANDRA[2], Giulio AIUDI[2]

[1]Società Veterinaria "Il Melograno" Srl, Sesto Calende, Varese, Italy;
[2]Dipartimento di Medicina Veterinaria (DiMeV), Section of Surgery an Obstetrics, University of Bari Aldo Moro, Italy.

e-mail: drsalvatorealonge@gmail.com; monica.melandri@email.it; leocivet@yahoo.it; giovannimichele.lacalandra@uniba.it; giulioguido.aiudi@uniba.it.

Corresponding Author:
Salvatore Alonge
Dipartimento di Medicina Veterinaria (DiMeV)
Sezione di Chirurgia e Ostetricia
University of Bari Aldo Moro, Italy.
Phone +39 392 8058524
e-mail: drsalvatorealonge@gmail.com

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Author contributions
All authors contributed to design the study, collect, analyze the data and draft the paper. All authors have approved the final version.

Conflict of interest
None of the authors of this article has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

SUMMARY

Local vessels ultrasonography evaluates prostatic physio-pathologic states. Testosterone promotes tissue and vascular growth. Knowing variables on prostatic vasculature is crucial to correctly apply Pulsed-Wave exam. The study aims to assess how ejaculation and blood testosterone affect Pulsed-Wave indexes. Serial blood testosterone dosages and Pulsed-Wave exams were performed in 20 dogs, immediately before (T0) and after (T1) ejaculation and 6 hours later (T2). *Arteria prostatica cranialis*, *Arteriola capsularis*, *Arteriola trabecularis* and *Arteriola parenchimalis* were evaluated and mean Pulsatility and Resistivity Index, Systolic-Peak, End-Dyastolic and Mean Velocity calculated. Data were grouped by time and vessel (ANOVA, $p \leq 0.05$). At T1, Resistivity Index significantly lowered in *Arteria prostatica cranialis*, *Arteriola trabecularis* and *Arteriola parenchimalis* but grew in *Arteriola capsularis*; Pulsatility Index had the same pattern, but not significant in *Arteriola parenchimalis;* Systolic Peak Velocity, End-Dyastolic Velocity, Mean Velocity significantly rose in *Arteriola capsularis* and *Arteriola trabecularis*. No indexes differed at T0 and T2. Testosterone did not differ at T0 (10.93±7.05ng/ml), T1 (12.71±7.29) and T2 (10.54±6.63). Results stated the risen prostatic vascular flow post ejaculation, affecting Pulsed-Wave. Due to semirigid capsule, impairing vasodilation of other vessels, only *Arteriola capsularis* indexes increased. Intimal cushions of *Arteria prostatica cranialis* kept velocities fixed; *Arteriola capsularis* and *Arteriola trabecularis* lack of intimal cushions, thus velocities grew. In *Arteriola parenchimalis*, pre-capillary sphincters opening allows increased flow redistribution in vasodilated parenchymal bed, keeping velocities fixed. Since testosterone, not affected by ejaculation, did not peak, vascular changes are not due to testosterone itself. These physiological effects of ejaculation suggest proper sexual rest before Pulsed-Wave exam planned to explore suspected prostatic neovascularization.

Keywords: dog, prostate, ultrasound, Pulsed-Wave, testosterone.

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INTRODUCTION

Ultrasonography is the most important tool to diagnose prostatic disorders (Mantziaras *et al.*, 2017). Normal and abnormal sonographic findings of the canine prostate have been well investigated (Russo *et al.*, 2012). However, no pathognomonic images to univocally diagnose any of the pathologic conditions by B-mode exam exist (Levy *et al.*, 2014). Thus, different Doppler techniques have been investigated to improve andrological diagnostics: Color, Power and Pulsed-Wave (PW) have been recently applied to the non-invasive study of local vascularization, to evaluate prostatic physio-pathological conditions, mainly considering increased blood flow during prostatic diseases (Günzel-Apel *et al.*, 2001; Freitas *et al.*, 2013; Zelli *et al.*, 2013). To make these techniques successful, appropriate knowledge of normal prostatic vascularization and deep identification of physiologic variables possibly affecting it are fundamental (Newell *et al.*, 1998). Modern imaging techniques necessitate additional names for prostatic arteries branches, resulting from anatomical microvasculature studies (Stefanov, 2004). *Arteria prostatica cranialis*, *media* and *caudalis* are muscle arteries, common blood distributing organic vessels, having intimal cushions (Stefanov, 2004). When reaching the gland, they branch, leaving *Arteriolae capsularis*, first-order arterioles, previously named Subcapsular arteries (Hodson, 1968), that form a single-plane net (Stefanov, 2004) in the semi-rigid prostatic capsule thickness, composed of fibro-elastic tissue (Rhodin, 1974). Second-order direct arterioles, *Arteriolae trabecularis* (Stefanov, 2004) and third-order branched arterioles, *Arteriolae parenchimalis*, first described by ultrasound contrast agent (Hagen *et al.*, 2000), provide parenchimal vascular supply. Testosterone, promoting androgenic-receptor-mediated tissue growth and stimulating angiogenesis and vascular growth (Franck *et al.*, 1998), must be considered among parameters possibly affecting prostatic vascularization. Recently, the role of ejaculation on prostatic vascular appearance on Power Doppler exam was assessed (Alonge *et al.*, 2018) in order not to misdiagnose the prostatic neovascularization occurring during glandular disorders (Newell *et al*., 1998; Freitas *et al*., 2013; Polisca *et al*., 2013; Zelli *et al*., 2013). Present

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study aimed to evaluate if and how ejaculation and blood testosterone affect prostatic Pulsed-Wave exam and to state how long this effect lasts.

MATERIALS AND METHODS

Twenty different-breed dogs were enrolled (2-5 y.o., 5-42 kg bodyweight), all healthy at physical examination and used to digital manipulation. The study was performed in accordance with animal welfare committee ethical guidelines and all procedures were carried according to the Italian legislation on animal care (DL 116, 27/01/1992; ethical approval n° 35/17 DETO, 26/06/2017). Informed owner consent was obtained. Sexual rest was required for 7 days before examination and during the study.

Serial PW prostate exams (MyLab™ClassC, Esaote Spa, Genua, Italy). and blood testosterone dosages (ELISA Testosterone, Demeditec Diagnostics GmbH, Kiel, Germany) were performed immediately before (T0) and after (T1) ejaculation, and 6 hours later (T2). In each exam, three PW images of *Arteria prostatica cranialis* (APC), *Arteriola capsularis* (AC), *Arteriola trabecularis* (AT) and *Arteriola parenchimalis* (AP) (Stefanov, 2004), with spectral tracing with three sequential waveforms (Figures 1-2-3-4), were evaluated. Medium values for Pulsatility (PI) and Resistivity (RI) Indexes, Systolic-Peak (SPV), End-Dyastolic (EDV) and Medium (MV) Velocities were calculated. Data were grouped according to time and vessel and statistically analyzed by ANOVA ($p \leq 0.05$).

RESULTS

At T1: RI significantly lowered in APC, AT and AP but grew in AC; PI had the same pattern, but not significant in AP; SPV, EDV, and MV significantly rose in AC and AT ($p \leq 0.05$). No indexes differed between T0 and T2. Specific results on PW parameters are reported in Table 1. Testosterone did not differ at T0 (10.93±7.05 ng/ml), T1 (12.71±7.29 ng/ml) and T2 (10.54±6.63 ng/ml).

DISCUSSION

Present results agree with previous reports, concerning basal PW exam (Newell *et al.*, 1998; Günzel-Apel *et al.*, 2001; Freitas *et al.*, 2013; Zelli *et al.*, 2013) as well as blood testosterone before and after ejaculation (Kobayashi *et al.*, 2013). To the authors' knowledge, PW prostatic parameters after ejaculation and prostatic vessels MV were never previously evaluated. Moreover, this is the first description by basal Doppler sonography of *Arteriola trabecularis*, whose flow was previously identified only by contrast-enhanced ultrasonography (Hagen *et al.*, 2000).

The increased vascular prostatic flow is detectable up to 24 hours after ejaculation by Power Doppler (Alonge *et al.*, 2018), which is very accurate in recognizing slow flows (Zelli *et al.*, 2013). Pulsed-Wave, more specific for high flows, is instead altered just for 6 hours. It can be inferred that ejaculation induces a definite increase in prostatic vascular provision, with high flows lasting for 6 hours, detectable by PW, and slow ones keeping on for 24 hours, highlighted by Power Doppler. Resistivity and Pulsatility Indexes increased only in AC due to semi-rigid capsular histology (Rhodin, 1974), not allowing vasodilation occurring in other vessels. Intimal cushions of APC (Stefanov, 2004), the only examined arteria, kept velocities constant. Conversely, AC and AT lack of intimal cushions, thus velocities increased. Finally, in AP the pre-capillary sphincters opening (Stefanov, 2004) allows the increased flow to redistribute in the vasodilated parenchymal vascular bed, keeping velocities constant.

Testosterone blood determination along time was not affected by ejaculation, thus vascular changes are not due to testosterone itself. Further studies should evaluate the role of other hormones, such as prostaglandins, primarily produced by sexual accessory glands (Kobayashi *et al.*, 2013), on prostatic ejaculative vascular changes.

Pulsed-Wave Doppler is a suitable non-invasive tool available to the clinician to study prostatic vascularization, fulfilling the morpho-functional evaluation of the organ. Present results indicate that, irrespective of basal patho-physiologic conditions, ejaculation alters PW appearance of the prostate. Thus, in conclusion, a proper sexual rest of at least 6 hours should be applied whenever a

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Pulsed-Wave exam is planned for a dog suspected of any prostatic disorders possibly leading to neovascularization.

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CONFLICT OF INTEREST

None of the authors of this article has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the paper content.

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Table 1. Pulsed-Wave Doppler parameters in each prostatic vessel (mean±SD).

PW	***Arteria prostatica cranialis***			***Arteriola capsularis***			***Arteriola trabecularis***			***Arteriola parenchimalis***		
Time	**T0**	**T1**	**T2**	**T0**	**T1**	**T2**	**T0**	**T1**	**T2**	**T0**	**T1**	**T2**
PI	3.323^{a} ±0.655	2.831^{b} ±0.632	3.040^{a} ^{b} ±0.714	1.033^{a} ±0.109	1.417^{b} ±0.314	1.259^{a} ^{b} ±0.487	1.413^{a} ±0.309	1.168^{b} ±0.238	1.519^{ab} ±0.618	0.939 ±0.149	0.856 ±0.225	0.910 ±0.309
RI	0.921^{a} ±0.026	0.874^{b} ±0.039	0.876^{b} ±0.038	0.606^{a} ±0.039	0.707^{b} ±0.066	0.675^{a} ^{b} ±0.077	0.708^{a} ±0.077	0.644^{b} ±0.064	0.714^{ab} ±0.115	0.578^{a} ±0.064	0.538^{b} ±0.083	0.556^{ab} ±0.130
SPV m/s	0.257 ±0.104	0.248 ±0.074	0.295 ±0.115	0.124^{a} ±0.038	0.227^{b} ±0.138	0.191^{a} ^{b} ±0.062	0.132^{a} ±0.040	0.161^{b} ±0.056	0.160^{ab} ±0.040	0.081 ±0.149	0.088 ±0.017	0.086 ±0.031
EDV m/s	0.021 ±0.009	0.030 ±0.012	0.036 ±0.019	0.050^{a} ±0.018	0.064^{b} ±0.030	0.058^{a} ^{b} ±0.010	0.037^{a} ±0.012	0.058^{b} ±0.023	0.043^{ab} ±0.020	0.034 ±0.013	0.039 ±0.011	0.040 ±0.022
MV m/s	0.073 ±0.023	0.077 ±0.028	0.089 ±0.026	0.073^{a} ±0.022	0.116^{b} ±0.059	0.109^{a} ^{b} ±0.041	0.068^{a} ±0.015	0.090^{b} ±0.027	0.079^{ab} ±0.021	0.049 ±0.020	0.057 ±0.013	0.055 ±0.025

Different superscripts denote statistically significant differences within rows for each parameter in specific vessel.

Figure 1. Speckle-tracking image of *Arteria prostatica cranialis* (APC).

Figure 2. Speckle-tracking image of *Arteriola capsularis* (AC).

Figure 3. Speckle-tracking image of *Arteriola trabecularis* (AT).

Figure 4. Speckle-tracking image of *Arteriola parenchimalis* (AP).

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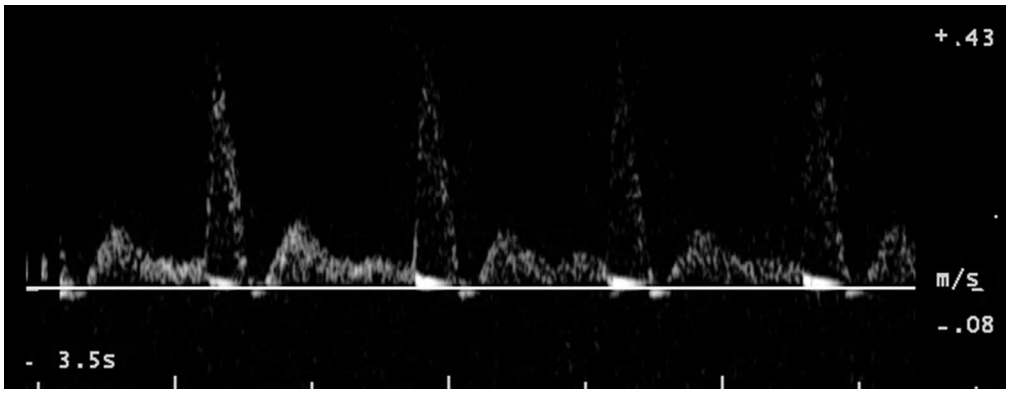

Figure 1. Speckle-tracking image of APC.

174x66mm (96 x 96 DPI)

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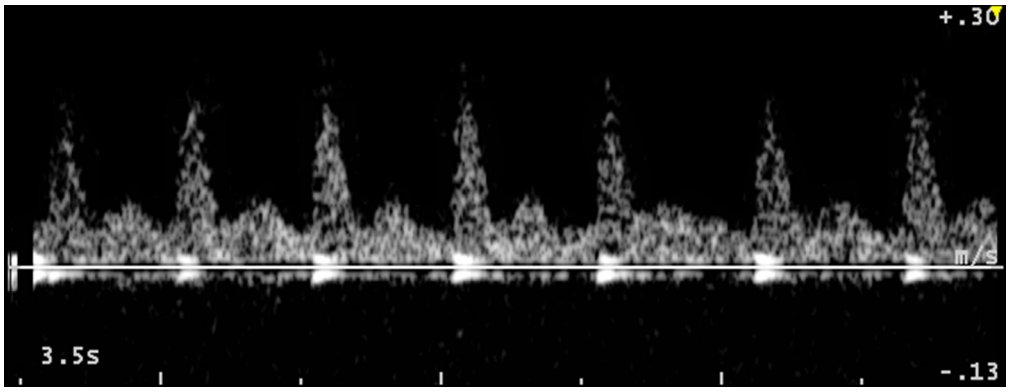

Figure 2. Speckle-tracking image of AC.

170x64mm (96 x 96 DPI)

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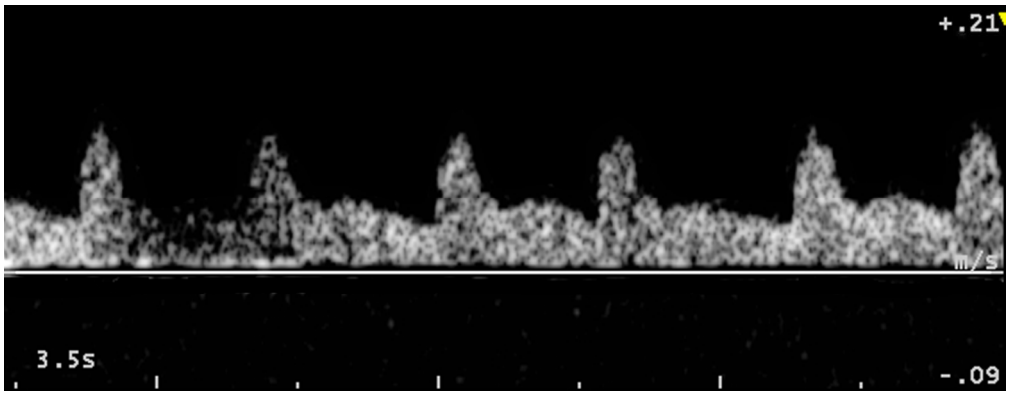

Figure 3. Speckle-tracking image of AT.

169x65mm (96 x 96 DPI)

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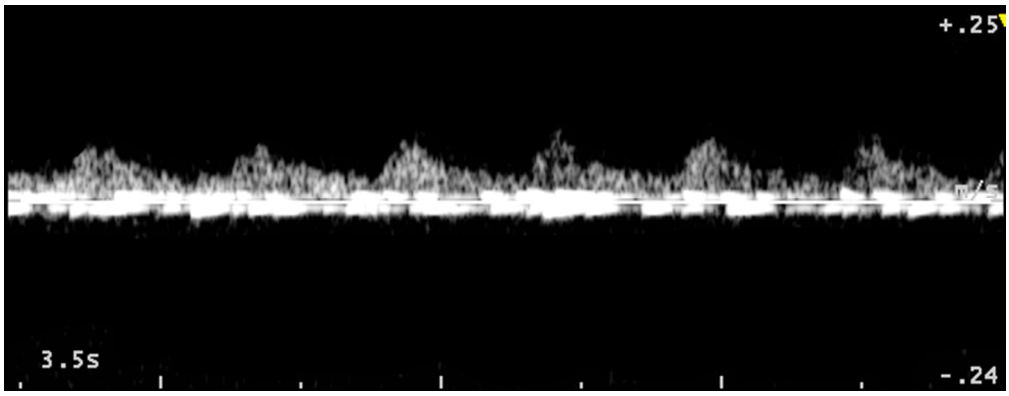

Figure 4. Speckle-tracking image of AP.

170x65mm (96 x 96 DPI)

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