DE GRUYTER OPEN

Bull Vet Inst Pulawy 58, 149-155, 2014
DOI: 10.2478/bvip-2014-0023

Dynamic tissue perfusion measurement in the reproductive organs of the female and male dogs

Michał Dzięcioł, Thomas Scholbach[1], Ewa Stańczyk, Justyna Ostrowska[2], Wojciech Kinda[2], Magdalena Woźniak[3], Wojciech Atamaniuk[2], Piotr Skrzypczak[2], Wojciech Niżański, Andrzej Wieczorek[3], Jacob Scholbach[4], Zdzisław Kiełbowicz[2]

Department of Reproduction and Clinic of Farm Animals, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, 50-366 Wroclaw, Poland
[1]Hospital for Children and Adolescents, Chemnitz Clinics, 4 Chemnitz, Germany
[2]Department of Surgery, 50-366 Wroclaw, Poland
[3]Department of Pediatric Radiology, Children`s Hospital, Medical University of Lublin, 20-059 Lublin, Poland
[4]Chameleon Software, Mathematisches Institut, Albert-Ludwigs Universitat Freiburg, 79-104, Freiburg, Germany
michaldzieciol@wp.pl

Received: July 9, 2013 *Accepted: February 11, 2014*

Abstract

The aim of this study was to investigate the usefulness of new software Pixel Flux (PXFX) for clinical evaluation of tissue perfusion in the field of reproduction in dogs. The experiment was performed on six adult Beagle dogs. Different organs and tissues of the animals were examined with the MyLab25 Gold ultrasound system. Blood flow in the ovary, testicle, prostate, the ramification of the penile artery, and the network of blood vessels of the pampiniform plexus were examined with the use of colour coded Doppler technique, and obtained data was evaluated with the PXFX software. The more objective digital evaluation of data obtained with colour Doppler sonography through the application of dynamic tissue perfusion measurements provides new opportunities for diagnosis, as well as continuous monitoring of the function of the examined tissues and organs. The use of PXFX software is strongly indicated as a tool in small animal practice as an additional method for evaluation of tissue perfusion, especially in the cases when other methods like pulsed wave Doppler techniques are difficult to be performed.

Key words: dogs, colour Doppler, blood flow, tissue perfusion, software, Pixel Flux.

Introduction

Analysis of blood flow with the Doppler technique is a useful method for evaluation of the physiological, as well as pathological condition of various tissues. Doppler techniques have been used in veterinary practice for many years, however, mostly the pulsed Doppler technique was applied (1, 4, 7, 13, 19-21, 23, 24, 39-41). Traditional methods allow for measurements of many important parameters, like peak systolic velocity (PSV), end-diastolic velocity (EDV), resistive index (RI), pulsatility index (PI), and the time-averaged maximum velocity (TAMA), which enable to describe and compare the blood flow into the particular organs. In many cases, because of the small size of the vessel, this kind of evaluation of tissue perfusion is not possible. In these cases the colour Doppler technique is recommended, which is based on assessment of the intensity of blood flow. Then, this intensity is analysed on the basis of brightness of colours, which reflect blood flow velocity. The colouring differs according to the velocity and direction of flow. Unfortunately, the analysis of the results obtained with this technique is difficult because it is subjective and gives no opportunity to evaluate reproducible, numerical values. Moreover, it cannot be evaluated with the statistic model.

New software Pixel Flux® (PXFX) is the first device which seems to eliminate the inconvenience of the aforementioned limitations of the colour Doppler technique. There are some examples describing the use of PXFX in veterinary literature, however, they focus

on large farm animals, and there is a lack of publications dedicated to small animals (15).

In this article, a principle of this new simple and accurate method for the evaluation of the perfusion of tissues in chosen organs in dogs is described.

Material and Methods

Location and animals. The study was conducted in the Department of Reproduction and in the Department of Surgery of the Faculty of Veterinary Medicine in Wroclaw University of Environmental and Life Sciences.

In six Beagle dogs, each three years old (two males and four females) blood flow was measured in the ramification of the penile artery, ovary, prostate, and network of blood vessels of the pampiniform plexus located in spermatic cord. All animals used in experiment belonged to the local Experimental Kennel. The study was approved by the Local Ethical Committee in Wrocław.

Equipment and procedures of examination. For ultrasonographic examinations of the animals, the MyLab25 Gold (Esaote, Italy) ultrasound system equipped with 12 MHz-micro convex probe and 12 MHz- linear probe was used. The dogs were examined in lateral recumbence and coupling gel was applied before examination. All dogs examined were familiar with these procedures, including prolonged examinations. In all cases sedative drugs were not used.

Software. Pixel Flux is a novel software, specifically designed for the dynamic measurement of tissue and organ perfusion analysis *via* colour Doppler ultrasound videos (34, 37).

The indispensable precondition for comparable results in perfusion measurements is the use of strictly standardised imaging planes, region of interest (ROI) and ultrasound machine presetting. Otherwise, no conclusions can be drawn from perfusion calculations. It is of utmost importance to define these conditions beforehand, to select the appropriate transsection of an organ or tissue, to take care of certain anatomical markers, which unanimously define the position of the organ with respect to the transducer, and to choose ROI with respect to the specific needs of the examination. Such standards cannot be overemphasised. Important parameters of the machine preset are transducer type, B-mode and colour Doppler frequencies, gain, filters, persistence, smoothing, edge, time or spatial resolution preferences, type of colour scale, imaging rate per second. At least one complete heart cycle must be captured within a video with a high number of images per second. PRF (pulse repetition frequency) and image scale can be changed, and are taken into account by the PXFX software.

The principle of the Pixel Flux-software is that each coloured pixel inside ROI is evaluated twice: its velocity value is decoded after comparison with the colour scale of the video, and its area is measured. Mean values of all velocity values and the coloured area inside the ROI are calculated for each single image of the entire video. Mean velocity and perfused area are multiplied and divided by the area of the entire ROI:

$I\ (cm/s) = v\ (cm/s) \times A\ (cm^2)/A\ ROI\ (cm^2)$;

I - mean flow intensity inside a ROI;

V - mean flow velocity of all coloured pixels inside the ROI;

A - area of all coloured pixels inside the ROI;

A ROI - area of the entire ROI.

Thus, it is calculated within the present single image. These calculations are repeated automatically for all images of the video. The Pixel Fluxsoftware then automatically recognises the beginning and end of complete heart cycles and takes into account complete heart cycles only. Thus, all measurements mirror the change of the basic Doppler parameters throughout complete heart actions. By referring all perfusion data to the entire ROI, encompassing also the non-perfused area, a much more realistic description of the tissue perfusion can be achieved than by single vessel investigation with the pulsed Doppler technique.

Over 40 useful parameters can be derived from the basic parameters V and A, most important of which is the I. All parameters are given separately, as well as combined for blue and red pixels. Among the frequently used parameters are the tissue resistance index (TRI) and tissue pulsatility index (TPI). These overcome the limitations of classic RI and PI, which refer to single vessel interrogations, and can be frequently misleading due to the fact, that in classical pulsed Doppler measurements only those vessels are subject to investigation, which are still coloured and thus visible. In vascular damage, a tissue is rarely perfused and vessels may be obliterated or only minimally perfused. Thus, they are missed in PW-interrogations, and a falsely positive impression of the overall tissue perfusion results. By referring to all vessels in the ROI and by taking into account the non-perfused vessel-free areas of the image, the novel TRI and TPI deliver a more realistic measure of the tissue perfusion. The Pixel Flux software also offers features for three-dimensional perfusion quantification with automatic simultaneous correction of all vessels' individual Doppler angles. Therefore, not only perfusion intensities, but also true flow volumes within a tissue can be measured.

Another useful feature is the overlay of the ROI with intensity false-colour maps based onto all local perfusion values of the entire video within a stationary map. Malperfused areas are visible at a first glance. Even more important is the numerical description of the vessel architecture within the ROI. Vessels are classified according to their perfusion intensity and the distribution of these vessel classes is diagrammatically displayed. Quartiles, mean, median, skewness and kurtosis are displayed as measures of the distribution curve, and transferred to the picture archiving and communication system (PACS). This distribution offers meaningful insight into the actual

state of the tissue's microvasculature. Chronic and acute affections of the microvessels become numerically evaluable.

The data is sent to an incorporated PACS, which is the platform for image and measurement comparison and review, and which can allow for export of single patient data and interindividual comparisons. All data is displayed in a variety of graphs and time-lines, and can be exported to spread-sheet and statistical software for rapid scientific evaluation. Figs 1 and 2 present a graphical analysis of blood flow in the examined organ (prostate).

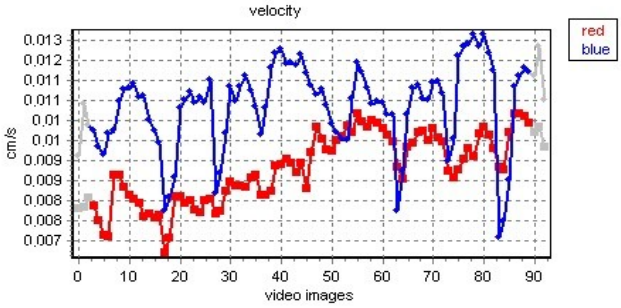


Fig. 1. Blood flow velocity in the vessel of canine prostate

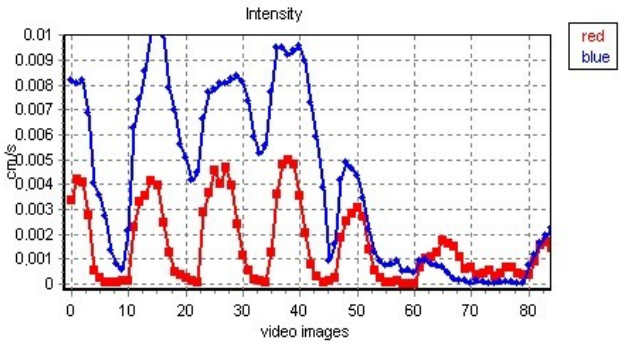


Fig. 2. Blood flow intensity, colours represents a direction of the blood flow (canine prostate)

Stepwise image analysis with the PixelFlux-technique: A video file (preferably DICOM format but avi-format works as well) is opened and the colour bar is read out and calibrated semi-automatically. Distances are also calibrated automatically (DICOM feature) and the ROI is defined according to the specific investigation by freehand, or by a predefined method, and thus is reproducible. Here, parallelograms with arbitrarily selectable and definable layers, or concentric circular, or freehand ROIs are offered. The measurement is carried out within a few seconds and can be automatised for a stack of video files for even faster and more convenient workflow (34, 35).

Results

In all animals, all organs and tissues planned for the evaluation were successfully examined. Images obtained by Colour Doppler projection, presenting the perfusion of the examined tissues, were digitally evaluated with the use of Pixel Flux software. The results are shown as separate charts presenting velocity or intensity of the blood flow (Figs 1 and 2), as images showing the perfusion of the particular tissues, and as charts presenting digital evaluation of the perfusion of tissue integrated with the original images (Figs 3-11).

Discussion

In human medicine, the Pixel Flux software is used in many medical specialties, including endocrinology, gastroenterology, gynaecology, hepatology, nephrology, neurology, oncology, paediatrics sonography, transplantation medicine, and urology (2, 8, 25, 28-33, 35-37, 42). In veterinary medicine till now, all available reports described the colour coded Doppler sonography, which takes into account the qualitative dynamic tissue perfusion measurements in farm animals (15).

In bitches, because of small size of the ovaries, it is difficult to examine them, and in some cases they cannot even be visualised. This fact makes the correct analysis of ovary perfusion very difficult with the use of traditional methods. On the other hand, the information obtained by the evaluation of changes in ovary perfusion could provide useful data about its actual functionality and activity. The importance of this kind of examination was previously suggested by authors examining the ovaries of mares and cows. Herzog *et al.* (13) even stated that "luteal blood flow is a more appropriate indicator for luteal function during the bovine oestrous cycle than luteal size". Miyamoto *et al.* (17) also concluded that the blood flow is a key regulatory component of the corpus luteum function. However, there is a couple of studies presenting blood flow in the bitches` ovaries, most of them focused on the intraovarian vessels, and not demonstrating the perfusion of the parenchyma in a quantitative manner (3, 14). The usefulness of this kind of analysis could be suspected, while there were some attempts for objective quantitative evaluation of the results obtained with the use of colour coded technique. In the report of Polisca *et al.* (22), the total number of colour pixels was calculated by a computer-assisted image analysis system using open source software ImageJ (Mac OS X, USA). This attempt at creating an objective evaluation method for the colour coded examination results confirms the need for a tool for that kind of digital analysis.

In our study, the ovaries of bitches were detected behind the caudal pole of the ipsilateral kidney. Fig. 3 presents the ovary perfusion obtained with the colour Doppler technique, showing a complex array of tiny vessels. Figure 4 presents the analysis of this view with the use of PXFX software.

The changes in blood flow in the vessels leading blood into the corpora cavernosa was described as one of the possible parameters which could be examined in the context of evaluation of the sexual arousal in male dogs.

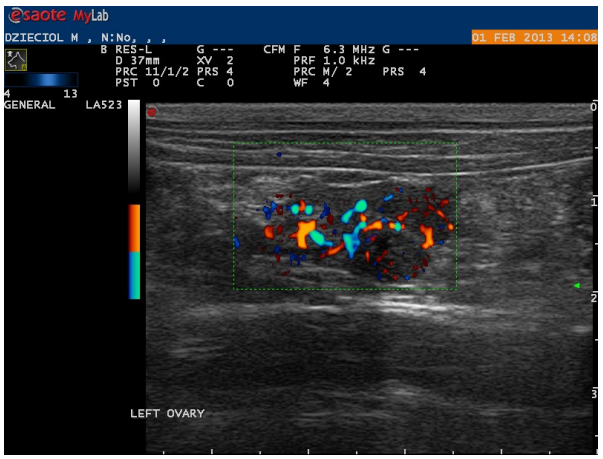


Fig. 3. Ovary perfusion - colour Doppler projection

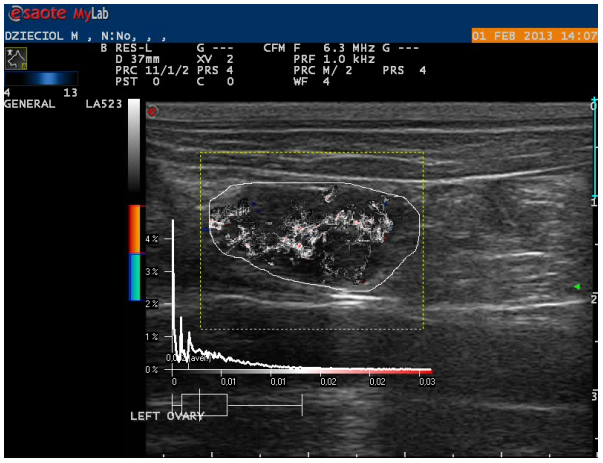


Fig. 4. Digital analysis of the ovary perfusion with the use of PXFX software

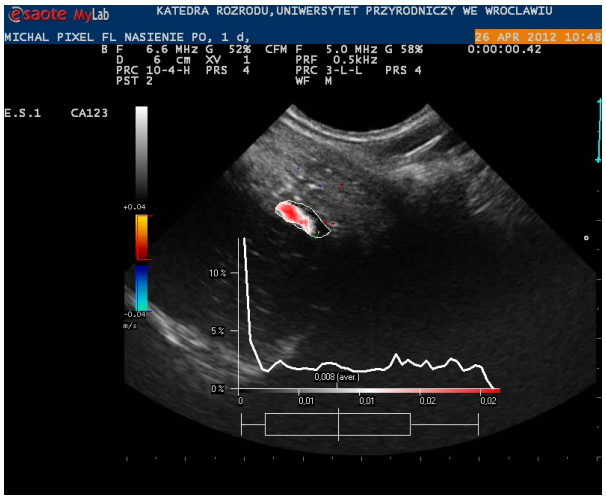


Fig. 5. Quantitative analysis of the changes of the blood flow in ramification of the penile artery after semiochemical stimulation

These changes occur as a consequence of natural semiochemical signals (sex pheromones) (9). The influence of the semiochemical signals (pheromones) on the erection in males has been also described in other species (26, 27). In our previous study, it was proven that blood flow in this small vessel changes due to pheromonal stimuli. However, the results of this evaluation could only be presented as a view of the vessel itself (filled-in to different degrees by blood, which could be seen *via* colour Doppler projection), but without the possibility of digital (objective) evaluation of the obtained results (9). With the use of Pixel Flux software, a detailed, quantitative analysis of the changes in the blood flow in this structure was possible (Fig. 5).

The usefulness of the Doppler sonography for the examination of canine testes is described in several reports (5, 11, 12). However, Pozor and McDonnell (23), who examined stallions` testes with the pulsed-wave gray-scale Doppler ultrasonography, found this method very helpful, despite much bigger size of the testes. The same authors concluded that even in stallions this method has its limitations, and explained that "identification of small vessels is difficult and evaluation of overall vascularisation of the parenchyma of stallion testis is usually not possible with this technology" (24). The mentioned authors also suggested that in some cases, the evaluation of perfusion of some vessels and/or organs is easier or just possible only with the use of the colour Doppler method. Figure 6 presents the perfusion of a healthy canine testicle.



Fig. 6. Digital analysis of the canine testicle

Nevertheless, the evaluation of the blood flow in canine testes was previously described, and the visualisation of the main bigger vessels usually does not cause trouble, in some cases the evaluation of the blood flow in the main vessel leading the blood into the entire organ is not sufficient. In the presence of pathological structures (*e.g.* tumour) located within the organ, the evaluation of perfusion of some fragments of parenchyma, or comparing perfusion of two chosen areas could bring additional important information. In these cases also the presence of data useful for objective digital evaluation could be indicated and helpful in the process of monitoring of, for example, the results of the some pharmacological treatment, especially since therapies involving impaired blood supply to tumour, and methods dedicated to tumor oxygenation are increasingly being used in medicine (6, 10). Figure 7 presents the perfusion of the dogs` testicular tissue with a tumour. Using the ROI, the analysis of the perfusion of different tissues –healthy and stricken by pathological condition can be evaluated and compared.

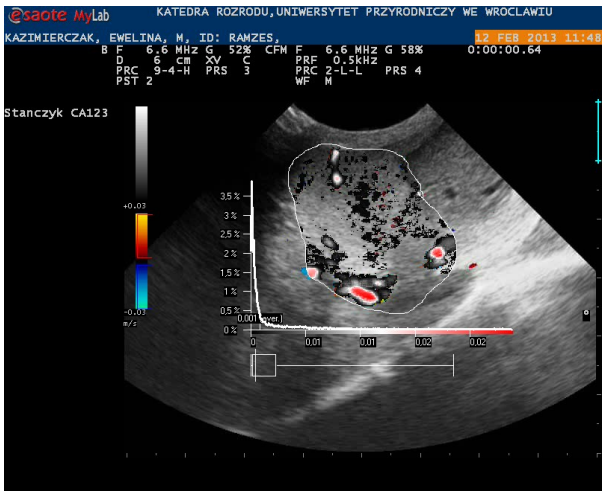


Fig. 7. The perfusion of the dogs` testicular tissue containing a tumour

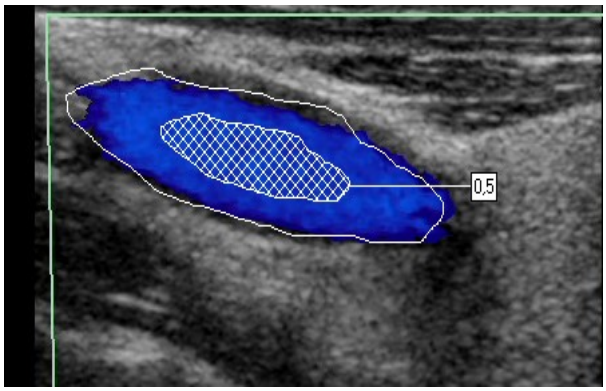


Fig. 8. Two separate regions of interest fixed on the view of the examined structure

It is worth mentioning that the unique function available in the Pixel Flux software also allows for simultaneous analysis of perfusion of two regions of the same organ. In these cases, two separate regions of interest could be fixed on the view of the examined structure, and blood flow could be calculated in these two regions at the same time (Fig. 8). This function could be also very useful for evaluation of perfusion of lymph nodes, especially during such pathological conditions as lymphoma, when the changes in blood flow within the core and cortical areas are present (17). This function could also be used for blood flow evaluation in the wall of the rural organs, such as the uterus or urethra (42).

The structure involved in proper functioning of male reproductive organs is the pampiniform plexus located in the spermatic cord. One of its roles is temperature regulation of the testes. In human, an abnormal enlargement of the pampiniform plexus is a medical condition called variocele. However, spontaneus variocele has not been diagnosed in dogs, while a secondary varicocele formation has been reported as a metastatis of Sertoli cell tumours (16). The pampiniform plexus contains a network of many small vessels, which makes the evaluation of the blood flow within this structure very difficult. The possibility of evaluation of the blood flow in the whole structure with the use of colour-coded sonography and not only in particular, short vessels (pulsed wave Doppler method) seems to be the optimal solution, which eliminates at the same time the potential problem of identifying the same vessel in a subsequent examination.

Figure 9 presents the perfusion of a dog's pampiniform plexus. Placing the net ROI (region of interest) over the vessels` allows for the examination of the whole area and evaluation of total perfusion observed in this region (Fig. 10).

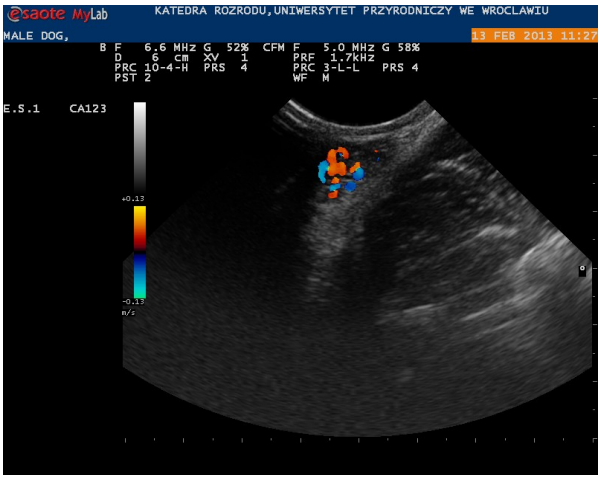


Fig. 9. The perfusion of a dog's pampiniform plexus

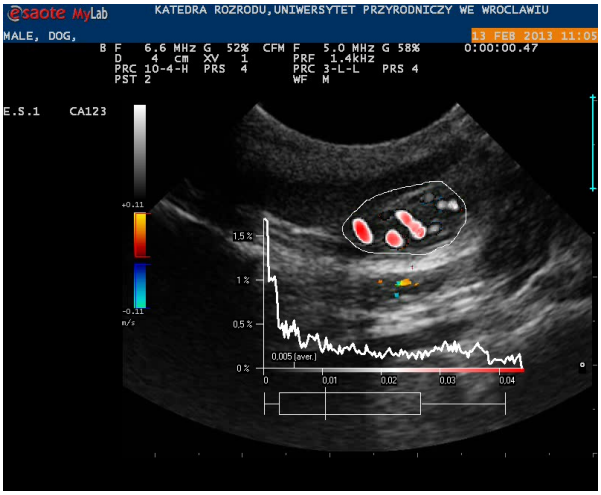


Fig. 10. Digital evaluation of the perfusion of a dog's pampiniform plexus

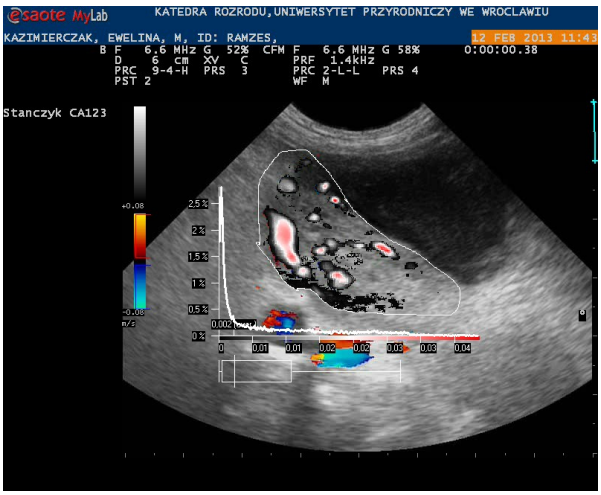


Fig. 11. Perfusion of one lobe of a pathologically changed prostate and its digital analysis

Another organ connected with the reproductive system of the males, which could be successfully examined with the use of colour coded Doppler sonography is the prostate. The size of that gland in dogs varies from 2 to 5 cm of diameter. The evaluation of blood flow in this organ can be challenging. Figure 11 presents the perfusion of one lobe of a pathologically changed prostate and its digital analysis.

In conclusion, it was demonstrated that the Pixel Flux software is a useful and modern tool dedicated to the quantification of the blood flow in tissues and entire organs. The results obtained surpass those from traditional pulsed Doppler technique investigations.

One of the most important limitations of colour-Doppler sonographic perfusion evaluation is its subjectivity. With the use of PXFX software, an objective digital evaluation and numerical description of a variety of tissue perfusion parameters, most importantly perfusion intensity, is now at hand. This also allows for a statistical evaluation of the obtained data. In our opinion, the use of this software in small animal veterinary practice is especially useful for examination of small parenchymal organs like the ovaries, lymph nodes, testes, and prostate. Other useful applications include vascular networks, such as the pampiniform plexus, perfusion of the walls of hollow organs, and perfusion measurements, as well as comparisons of different ROIs within the same organ (*e.g.* monitoring the perfusion of the tumours).

References

1. Alvarez–Clau A., Liste F.: Ultrasonographic characterization of the uterine artery in the nonestrus bitch. Ultrasound Med Biol 2005, 3112, 1583–1587.
2. Aslan M.I., Kucukaslan S., Mulazimoglu T., Soyer M., Senyucel M., Cakmak J., Scholbach T., Aslan S.: Quantitative software analysis of ultrasonographic textures in experimental testicular torsion. Eur J Pediatr Surg 2013, 23, 134–139.
3. Bergeron L.H., Nykamp S.G., Brisson B.A., Madan P., Gartley C.J.: An evaluation of B–mode and color Doppler ultrasonography for detecting periovulatory events in the bitch Theriogenology 2013, 79, 274–283.
4. Brozos C.N., Pancarci M.S., Valencia J., Beindorff N., Tsousis G., Kiossis E., Bollwein H.: Effect of oxytocin infusion on luteal blood flow and progesterone secretion in dairy cattle J Vet Sci 2012, 13, 67–71.
5. Carrillo J.D., Soler M., Lucas X., Agut A.: Colour and pulsed Doppler ultrasonographic study of the canine testis. Reprod Domest Anim 2012, 47, 655–659.
6. Di Martino E., Gagel B., Schramm O., Maneschi P., Westhofen M.: Evaluation of tumor oxygenation by color duplex sonography: A new approach. Otolaryng Head Neck 2005, 132, 765–769.
7. Di Salvo P., Bocci F., Zelli R., Polisca, A.: Doppler evaluation of maternal and fetal vessels during normal gestation in the bitch Res Vet Sci 2006, 81, 382–388.
8. Dubinsky T., Chen P., Maklad N.: Color–flow and power Doppler imaging of the testes. World J Urol 1998, 16, 35–40.
9. Dzięcioł M., Stańczyk E., Noszczyk–Nowak A., Niżański W., Ochota M., Kozdrowski R.: Influence of bitches sex pheromones on the heart rate and other chosen parameters of blood flow in stud dogs (*Canis familiaris*). Res Vet Sci 2012, 93, 1241–1247.
10. Gagel B., Piroth M., Pinkawa M., Reinartz P., Zimny M., Kaiser H.J., Stanzel S., Asadpour B., Demirel C., Hamacher K., Coenen H.H., Scholbach T., Maneschi P., DiMartino E., Eble M.J.: pO polarography, contrast enhanced color duplex sonography (CDS), [^{18}F] fluoromisonidazole and [^{18}F] fluorodeoxyglucose positron emission tomography: validated methods for the evaluation of therapy–relevant tumor oxygenation or only bricks in the puzzle of tumor hypoxia? BMC Cancer 2007, 7, 113.
11. Günzel–Apel A.R., Möhrke C., Poulsen–Nautrup C.: Colour–coded and pulsed Doppler sonography of the canine testis, epididymis and prostate gland: physiological and pathological findings. Reprod Domest Anim 2001, 36, 236–240.
12. Gumbsch P., Holzmann A., Gabler C.: Colour–coded duplex sonography of the testes of dogs. Vet Rec 2002, 151, 140–144.
13. Herzog K., Brockhan–Lüdemann M., Kaske M., Beindorff N., Paul V., Niemann H., Bollwein H.: Luteal blood flow is a more appropriate indicator for luteal function during the bovine estrous cycle than luteal size. Theriogenology 2010, 73, 691–697.
14. Köster K., Poulsen–Nautrup C., Günzel–Apel A.R.: Doppler ultrasonographic study of cyclic changes of ovarian perfusion in the Beagle bitch, Reproduction 2001, 122, 453–461.
15. Kuchler K., Sauter–Louis C., Martin R., Mansfeld R.: Measuring the blood flow of teats of dairy cows by using Color–Angiography. Reprod Domest Anim 2011, 46, Suppl, 1, 25.
16. McNeil P.E., Weaver A.D.: Massive scrotal swelling in two unusual cases of canine Sertoli cell tumour. Vet Rec 1980, 106, 144–146.
17. Miyamoto A., Shirasuna K., Wijayagunawardane M.P., Watanabe S., Hayashi M., Yamamoto–Matsui MD., Acosta T.J.: Blood flow: a key regulatory component of corpus luteum function in the cow. Domest Anim Endocrinol 2005, 29, 329–339.
18. Nakase K., Yamamoto K., Hiasa A., Tawara I., Yamaguchi M., Shiku, H.: Contrast–enhanced ultrasound examination of lymph nodes in different types of lymphoma. Cancer Detect Prev 2006, 30, 188–191.
19. Ostrowska J.: Resistivity index (RI) estimated by means of Doppler ultrasonography in renal interlobar arterie in dogs and cats with kidney diseases. PhD Thesis, University of Environmental and Life Sciences, Wroclaw, 2013.
20. Pasławska U., Noszczyk–Nowak A., Janiszewski A., Nicpoń J.: Tricuspid dysplasia in dogs. Bull Vet Inst Pulawy 2013, 57, 123–126.
21. Pasławska U., Noszczyk–Nowak A., Nicpoń J.: An assessment of left ventricular systolic function with the use of a continuous Doppler imaging in dogs with spontaneous mitral regurgitation. A preliminary study. Bull Vet Inst Pulawy 2012, 56, 399–402.
22. Polisca A., Zelli R., Troisi A., Orlandi R., Brecchia G., Boiti C.: Power and pulsed Doppler evaluation of ovarian hemodynamic changes during diestrus in pregnant and nonpregnant bitches. Theriogenology 2013, 79, 219–224.
23. Pozor M.A., McDonnell S.M.: Doppler ultrasound measures of testicular blood flow in stallions. Theriogenology 2002, 58, 437–440.
24. Pozor M.A., McDonnell S.M.: Color Doppler ultrasound evaluation of testicular blood flow in stallions. Theriogenology 2004, 61, 799–810.
25. Rosenbaum C., Wach S., Kunath F., Wullich B., Scholbach T., Engehausen D.G.: Dynamic tissue perfusion measurement: a new tool for characterizing renal perfusion in renal cell carcinoma patients. Urol Int 2013, 90, 87–94.
26. Sachs B.D.: Erection evoked in male rats by airborne scent from estrus females. Physiol Behav 1997, 62, 921–924.
27. Sachs B.D., Akasofu K., Citron J.H., Daniels S.B., Natoli J.H.: Noncontact stimulation from estrus females evokes penile erection in rats. Physiol Behav 1994 55, 1073–1079.
28. Scholbach T., Dimos I., Scholbach J.: A new method of color Doppler perfusion measurement *via* dynamic sonographic signal quantification in renal parenchyma. Nephron Physiol 2004, 96, 99–104.

29. Scholbach T., Herrero I., Scholbach J.: Dynamic color Doppler sonography of intestinal wall in patients with Crohn disease compared with healthy subjects. J Pediatr Gastr Nutr 2004, 39, 524–528.
30. Scholbach T., Girelli E., Scholbach J.: Dynamic tissue perfusion measurement: a novel tool in follow–up of renal transplants. Transplantation 2005, 79, 1711–1716.
31. Scholbach T., Scholbach J., Krombach G.A., Gagel B., Maneschi P., Di Martino E.: New method of dynamic Color Doppler signal quantification in metastatic lymph nodes compared to direct polarographic measurements of tissue oxygenation. Int J Cancer 2005, 114, 957–962.
32. Scholbach T., Girelli E., Scholbach J.: Tissue pulsatility index: a new parameter to evaluate renal transplant perfusion. Transplantation 2006, 81, 751–755.
33. Scholbach T.: From the nutcracker–phenomenon of the left renal vein to the midline congestion syndrome as a cause of migraine, headache, back and abdominal pain and functional disorders of pelvic organs. Med Hypotheses 2007, 68, 1318–1327.
34. Scholbach T., Scholbach J.: Dynamic sonographic tissue perfusion measurement. J Med Ultrasound 2009, 17, 71–85.
35. Scholbach T., Hormann J., Scholbach J.: Dynamic tissue perfusion measurement in the intestinal wall–correlation with ulcerative colitis. J Med Ultrasound 2010, 18, 62–70.
36. Scholbach T.M., Stolle J., Scholbach J.: Three–dimensional volumetric spatially angle–corrected pixelwise fetal flow volume measurement: Ultraschall Med 2011. 32 Suppl 2, 122–128.
37. Scholbach T.: Dynamic tissue perfusion measurement – Basics and Applications In: *Sonography*, Kerry Thoirs (editor), InTech, Croatia, 2012.
38. Scholbach T.M., Konje J., Huppertz B.: Pixelwise quantification of placental perfusion visualized by 3D power Doppler sonography. Ultraschall Med 2012, 33, 88–94.
39. Scotti L., Di Salvo P., Bocci F., Pieramati C., Polisca A.: Doppler evaluation of maternal and foetal vessels during normal gestation in queen. Theriogenology 2008, 69, 1111–1119.
40. Stańczyk E., Antonczyk A., Dzieciol M., Twardon J., Nizanski W.: Assessment of resistive indices of testicular artery as predictors of spermatogenesis in dogs. Reprod Dom Anim 2012, 47, Suppl 2, 49–49.
41. Starke A., Schmidt S., Haudum A., Scholbach T., Wohlsein P., Beyerbach M., Rehage J.: Evaluation of portal blood flow using transcutaneous and intraoperative Doppler ultrasonography in dairy cows with fatty liver. J Dairy Sci 2011, 94, 2964–2971.
42. Wieczorek A.P., Wozniak M., Stankiewicz A., Santoro G.A., Bogusiewicz M., Rechberger T., Scholbach J.: Quantitative assessment of urethral vascularity innulliparous females using high–frequency endovaginal ultrasonography. World J Urol 2011, 29, 625–628.