Animal Reproduction Science 141 (2013) 172–176

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Doppler ultrasonographic assessment of uterine arteries during normal canine puerperium

P.R. Batista[a,*], C. Gobello[b], Y. Corrada[b], E. Pons[c], D.O. Arias[a], P.G. Blanco[a]

[a] Cardiology Laboratory, Faculty of Veterinary Sciences, National University of La Plata (FVS-NULP), 60 y 118, La Plata 1900, Argentina
[b] Laboratory of Reproductive Physiology, FVS-NULP, La Plata, Argentina
[c] Imaging Diagnosis Service, FVS-NULP, La Plata, Argentina

ARTICLE INFO

Article history:
Received 20 December 2012
Received in revised form 1 July 2013
Accepted 30 July 2013
Available online 6 August 2013

Keywords:
Doppler
Ultrasound
Postpartum
Dog
Uterus
Blood flow

ABSTRACT

The aim of this study was to describe Doppler ultrasonographic changes of uterine arteries during normal canine puerperium. Eight healthy, pure-bred bitches, were ultrasonographically assessed during the postpartum period on Days −3, 3, 10, 17, 24, 38, 52 and 80 (Day 0 defined as the day of parturition). Total horn diameters (TD) and endometrium thickness (*E*) were evaluated. Color Doppler was used to localize uterine arteries at both sides of the body and pulsed-wave Doppler was performed to obtain the waveforms. Peak systolic velocity (PSV) and end diastolic velocity (EDV) were measured. Resistance index [(PSV − EDV)/PSV] was automatically calculated.

Values of TD, *E*, PSV, EDV and RI were analyzed by repeated measures ANOVA followed by LSD test (SPSS 18.0; SPSS, Chicago, IL, USA). A correlation analysis was also carried out between RI and TD. A progressive decrease of TD ($P<0.01$) and *E* ($P<0.01$) was found in the course of the study. A gradual diminution of PSV ($P<0.01$) and EDV ($P<0.01$) and an increase of RI ($P<0.01$) were also found throughout the study period. The resistance index negatively correlated with TD ($r=-0.46$; $P<0.01$) and *E* ($r=-0.44$; $P<0.01$) while the ultrasonographic and vascular changes in this period are concurrent with regenerative changes in the glandular and epithelial structures of the uterus. It is concluded that uterine artery RI progressively increased during normal canine puerperium, associated to the two-dimensional ultrasonographic regression of the organ.

1. Introduction

In mammalian species, the puerperium is a physiological period during which the female reproductive tract prepares to return to cyclicity (Orfanou et al., 2009; Mulic-Lutvica et al., 2001). In dogs, the vulvar discharge indicative of clinical pueperium is present for 4–7 weeks after parturition (Orfanou et al., 2008; Feldman and Nelson, 2004; Dickie and Arbeiter, 1993). Furthermore, the presence of placental sites and trophoblast-like cells has been considered a normal feature of uterine involution up to Day 84 after whelping (Al-Bassam et al., 1981; Orfanou et al., 2009).

In canine species, there are only two ultrasonographic reports of normal uterine involution during puerperium. Pharr and Post (1992) who described these changes during the first 24 days after whelping and Yeager and Concannon (1990) who studied uterine shape, echogenicity and diameter during 15 weeks. Immediately after whelping, the uterus was enlarged and echogenic presenting thick and irregular walls (Pharr and Post, 1992). Following this prolonged gradual involution of the uterus, which exceeds previous histological reports (Al-Bassam et al., 1981), was found (Yeager and Concannon, 1990).

Doppler ultrasound is a non invasive technique that provides information about the characteristics of the arterial blood flow and the organ perfusion (Fielder and Baker,

* Corresponding author. Tel.: +54 221 423 6663; fax: +54 221 427 7378.
E-mail addresses: pbatista@fcv.unlp.edu.ar, prbatista.mv@gmail.com (P.R. Batista).

0378-4320/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.anireprosci.2013.07.013

1969). This technique has been used to evaluate uterine arteries blood flow in many species, including dogs (Blanco et al., 2008). In bitches, the resistance index (RI) of this artery decreases during normal (Nautrup, 1998; Di Salvo et al., 2006; Blanco et al., 2011a,b) but not in abnormal gestations (Blanco et al., 2011a,b).

Uterine vascular changes during puerperium have been reported in women (Jaffa et al., 1996; Mulic-Lutvica et al., 2007), cows (Krueger et al., 2009) and mares (Mortensen et al., 2011). In these species, a progressive increase in RI was found throughout the postpartum period. Conversely, uterine arteries RI does not change after parturition in women showing puerperal endometritis, uterine subinvolution, delayed placental separation or impaired wound healing after C-section (Kirkinen et al., 1988; Nakai et al., 1997; Mulic-Lutvica et al., 2009).

Although characterization of postpartum uterine blood flow by Doppler ultrasound will probably contribute to the precocious diagnosis of postpartum diseases, this technique has not been used in puerperal bitches thus far. Therefore, the aim of this study was to describe Doppler ultrasonographic changes in uterine arteries during normal canine puerperium.

2. Materials and methods

2.1. Animals

Eight clinically healthy, 3.25 ± 0.25 (1–5) year-old, purebreed (5 Miniature Poodle, 1 Yorkshire Terrier, 1 Chihuahua and 1 Bichon Frise) pregnant bitches, weighing 3.68 ± 0.38 (2.5–6) kg were included in this study. Pregnancy was confirmed by two-dimensional ultrasonographic examination in all the cases (Day −3; England et al., 2003). The females were multiparous and had a history of normal whelping.

2.2. Ultrasonographic follow up

The bitches were ultrasonographically evaluated on Day −3 and then on Days 3, 10, 17, 24, 38, 52 and 80 after delivery. Day 0 was defined as the day of parturition. Two-dimensional and Doppler ultrasonographic evaluations were carried out with a 7.5–8 MHz linear transducer (Toshiba Core Vision Pro, Japan). The dogs were positioned in lateral recumbency, acoustic gel was applied to the transducer and coupled directly to the clipped skin. The uterine body was observed with two-dimensional ultrasound in a transversal axis (Davidson and Baker, 2009). At each evaluation time point after whelping, both uterine horns were assessed immediately after body bifurcation (Yeager and Concannon, 1990).

Total diameter (TD; mm) and endometrium thickness (*E*; mm) were measured in the transverse section of the uterine horn (Yeager and Concannon, 1990). The endometrium was identified as a moderately echogenic ring, which was surrounded by a hypoechoic ring of myometrium (Pharr and Post, 1992).

Color Doppler was used to localize uterine arteries at both sides of the uterine body and pulsed-wave Doppler was performed to obtain the waveforms (Alvarez-Clau and Liste, 2005). The angle of insonation was manually corrected according to previous reports (Köster et al., 2001; Di Salvo et al., 2006; Scotti et al., 2008 and Polisca et al., 2010). Measurements with an angle <20° were only considered for analysis. Three consecutive waveforms with maximum Doppler shift were included in the study (Köster et al., 2001). Peak systolic velocity (PSV; cm/s) and end diastolic velocity (EDV; cm/s) were measured while RI [(PSV − EDV)/PSV] was automatically calculated (Dickey, 1997).

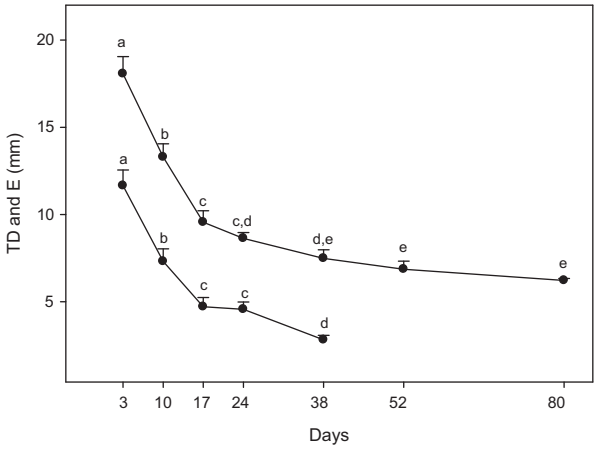

Fig. 1. Total uterine diameter (TD; mean ± SEM; solid circles) and endometrium thickness (*E*; mean ± SEM; empty circles) of eight female dogs during normal puerperium. For each variable, different letters indicate differences ($P < 0.05$) among days.

2.3. Statistical analysis

Values of TD, *E*, PSV, EDV and RI were analyzed by repeated measures ANOVA followed by LSD test (SPSS 19.0; SPSS, Chicago, IL, USA). To further interpret the findings, RI was correlated with both TD and *E* using Pearson's correlation test. $P < 0.05$ was considered significant.

3. Results

All the females whelped normally (2–5 healthy puppies), had an uneventfully clinical puerperium (Orfanou et al., 2008) and weaned their puppies on Day 60. Both TD ($P < 0.01$) and *E* ($P < 0.01$) progressively decreased in the course of the puerperium (Fig. 1). Endometrium was ultrasonographically observed up to Day 38. No differences were found between right and left PSV ($P > 0.1$), EDV ($P > 0.1$) and RI ($P > 0.1$), therefore values were averaged. Original recording of pulsed-wave Doppler ultrasound of the left uterine artery is shown in Fig. 2. A gradual diminution of PSV ($P < 0.01$; Fig. 3) and EDV ($P < 0.01$; Fig. 4) and an increasing RI ($P < 0.01$; Fig. 5) were also found throughout the study period. Resistance index was negatively correlated with TD ($r = -0.46$; $P < 0.01$) and *E* ($r = -0.44$; $P < 0.01$).

4. Discussion

In the course of this study, uterine dimensions and blood flow progressively decreased, indicating a normal

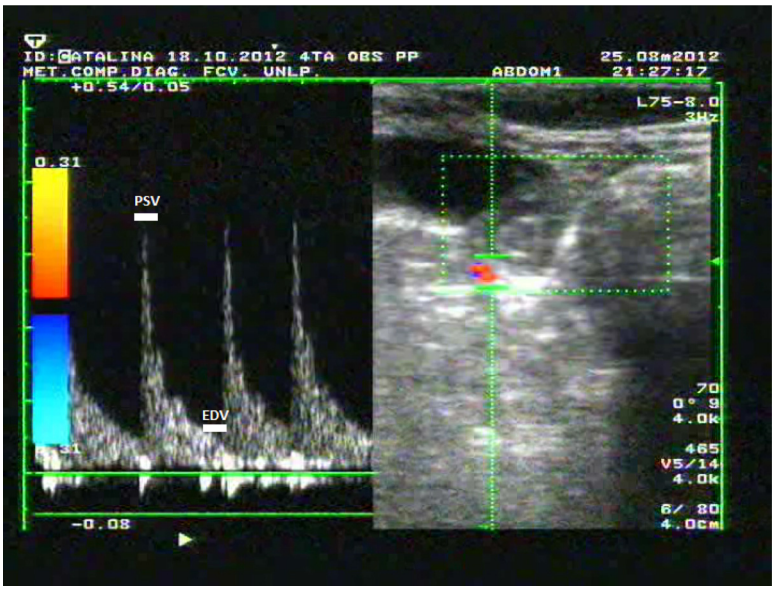

Fig. 2. Pulsed-wave Doppler ultrasound register of uterine artery in a selected pregnant bitch 24 days after parturition. The cursor was positioned on the artery. PVS is the peak systolic velocity and EDV the end diastolic velocity.

involution of the organ throughout puerperium. These findings are concomitant with the regenerative uterine changes in glandular and epithelial structures previously described for dogs after parturition (Orfanou et al., 2009; Chu et al., 2002). Uterine horns diameter diminished up to Day 38, which is in line with a previous report in dogs (Yeager and Concannon, 1990). Conversely, endometrial layer was ultrasonographically distinguished up to Day 52. According to a microscopic study in this species, placental site involution is almost complete at the beginning of the eighth week (Day 56) after parturition (Al-Bassam et al., 1981) at which the endometrium is lined by a single layer of foamy columnar epithelial cells. This histological description justifies the inability to ultrasonographically detect the endometrium after Day 52 in the present study. Conversely, Yeager and Concannon (1990) was able to visualize this layer up to Day 84 in four out of five Beagle bitches.

In this study, ultrasonographic appearance of the canine uterus became indistinguishable from that of anestrus the last two examinations (Days 52 and 80), while Yeager and Concannon (1990) found this hypoechoic image beyond Day 100. These variations in endometrium and uterine ultrasound involution between studies may be due to the differences in maternal weight and litter size between reports. The present results seem to confirm that uterine ultrasonographic involution occurs before that previously described in histological studies (Day 84; Al-Bassam et al., 1981; Orfanou et al., 2009).

As expected, in these animals uterine blood flow progressively decreased during normal puerperium. Peak systolic velocity and EDV of uterine artery diminished in

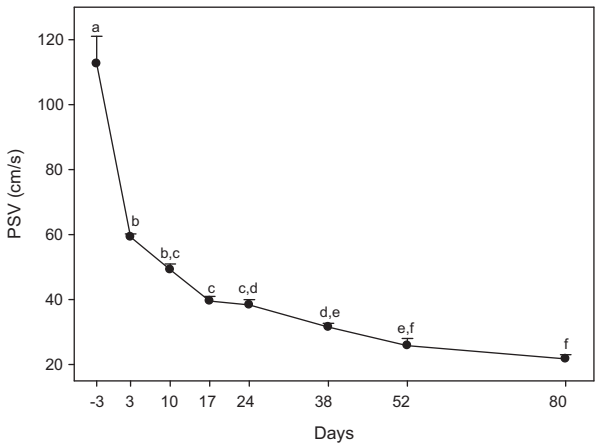

Fig. 3. Peak systolic velocity (PSV; mean ± SEM) of the same dogs of Fig. 1. Different letters indicate differences ($P < 0.05$) among days.

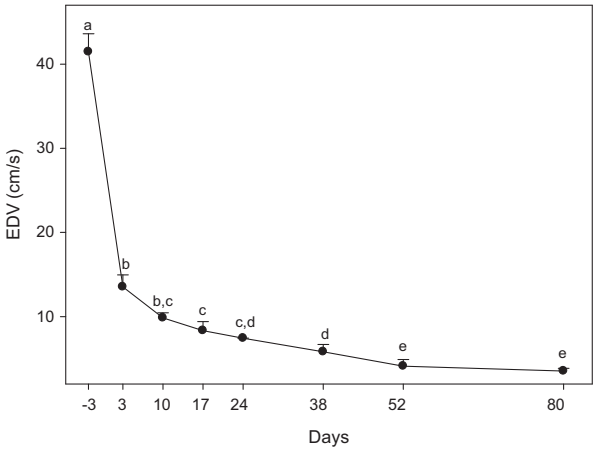

Fig. 4. End diastolic velocity (EDV; mean ± SEM) of the same dogs of Fig. 1. Different letters indicate differences ($P < 0.05$) among days.

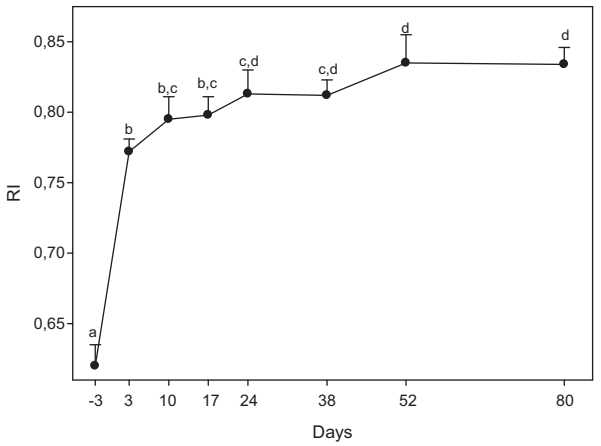

Fig. 5. Resistance index (RI) of uterine artery (mean ± SEM) of the same dogs of Fig. 1. Different letters indicate differences ($P < 0.05$) among days.

the course of the study, while RI increased. The resistance index increase is consistent with a previous report in dogs (Batista et al., 2012) and also in other species (Jaffa et al., 1996; Mulic-Lutvica et al., 2007; Krueger et al., 2009; Mortensen et al., 2011), in which RI increased markedly during the first days postpartum and then moderately up to involution. An explanation for this biphasic decrease in the uterine blood flow is the abrupt decrease of the uterine size that occurs the first week after parturition.

The outward hypertrophic remodeling of the uterine artery is a gestational event characterized by the artery luminal increase that has been described in rats (Cipolla and Osol, 1994), mice (van der Heijden et al., 2005) and sheep (Annibale et al., 1989). The involution of this arterial hypertrophy is a consequence of endothelial cells apoptosis in mice that leads to a decrease in uterine artery blood flow (van der Heijden et al., 2009). A similar process might also be present in dogs, although it remains to be described.

Despite the fact that the uterine artery RI remained unchanged from Day 24 to the end of the experiment, in this period, it was lower than values previously reported for anestrous dogs (Alvarez-Clau and Liste, 2005), suggesting a slow return to the non-pregnant conditions. A similar situation can be observed in women and cows, where increased peripheral resistance of uterine arteries exceeds clinical uterine involution (Tekay and Jouppila, 1993; Jaffa et al., 1996; Krueger et al., 2009).

Finally, Doppler ultrasound seems to be a useful tool for assessing changes in uterine artery blood flow during physiological canine puerperium and, therefore, for the early detection of postpartum disorders. It is concluded that the uterine artery RI progressively increased during normal canine puerperium which is associated with the two-dimensional ultrasonographic regression of the organ. Further work is still necessary to evaluate the influence of litter size, maternal age and weight, parity, lactation and C-section on postpartum remodeling process.

Acknowledgments

This study was partially funded by the University Incentive Program of Teaching and Research 11/V195. The authors are Career Scientists (CG, YC and PGB) and Research Fellow (PRB) of the National Research Council (CONICET) of Argentina.

References

Al-Bassam, M.A., Thompson, R.G., O'Donell, L., 1981. Normal postpartum involution of the uterus in the dog. Can. J. Comp. Med. 45, 217–232.
Alvarez-Clau, A., Liste, F., 2005. Ultrasonographic characterization of the uterine artery in the nonestrus bitch. Ultrasound Med. Biol. 3, 583–587.
Annibale, D.J., Rosenfeld, C.R., Kamm, K.E., 1989. Alterations in vascular smooth muscle contractility during ovine pregnancy. Am. J. Physiol. 256, 1282–1288.
Batista, P.R., Blanco, P.G., Tórtora, M., Arias, D.O., Corrada, Y.A., Gobello, C., 2012. Doppler ultrasonographic assessment of uterine arteries during normal canine puerperium. In: Proceedings of the 7th International Symposium on Canine and Feline Reproduction, Whistler, Canada, pp. 17–18.
Blanco, P.G., Arias, D.O., Gobello, C., 2008. Doppler ultrasound in canine pregnancy. J. Ultrasound Med. 27, 1745–1750.
Blanco, P.G., Rodríguez, R., Rube, A., Arias, D.O., Tórtora, M., Díaz, J.D., Gobello, C., 2011a. Doppler ultrasonographic assessment of maternal and fetal blood flow in abnormal canine pregnancy. Anim. Reprod. Sci. 126, 130–135.
Blanco, P.G., Tórtora, M., Rodríguez, R., Arias, D., Gobello, C., 2011b. Ultrasonographic assessment of maternal cardiac function and peripheral circulation during normal gestation in dogs. Vet. J. 190, 154–159.
Chu, P.Y., Salamonsen, L.A., Lee, C.S., Wright, P.J., 2002. Matrix metalloproteinases (MMPs) in the endometrium of bitches. Reproduction 123, 467–477.
Cipolla, M., Osol, G., 1994. Hypertrophic and hyperplastic effects of pregnancy on the rat uterine arterial wall. Am. J. Obstet. Gynecol. 171, 805–811.
Davidson, A.P., Baker, T.W., 2009. Reproductive ultrasound of the bitch and queen. Top. Companion Anim. Med. 24, 55–63.
Di Salvo, P., Bocci, F., Polisca, A., 2006. Doppler evaluation of maternal and fetal vessels during normal gestation in the bitch. Res. Vet. Sci. 81, 382–388.
Dickey, R.P., 1997. Doppler ultrasound investigation of uterine and ovarian blood flow in infertility and early pregnancy. Hum. Reprod. Update 3, 467–503.
Dickie, M.B., Arbeiter, K., 1993. Diagnosis and therapy of the subinvolution of placental sites in the bitch. J. Reprod. Fertil. Suppl. 47, 471–475.
England, G., Yeager, A., Concannon, P.W., 2003. Ultrasound imaging of the reproductive tract of the bitch. In: Concannon, P.W., Verstegen, J., England, G. (Eds.), Recent Advances in Small Animal Reproduction. IVIS, Ithaca, NY (http://www.ivis.org), document A1226.0303.
Feldman, E.C., Nelson, R.W., 2004. Breeding, pregnancy and parturition. In: Canine and Feline Endocrinology and Reproduction. Saunders, Philadelphia, pp. 775–776.
Fielder, F.D., Baker, R.F., 1969. Diagnostic applications of Doppler ultrasound. Ultrasonics 7, 36–38.
Jaffa, A.J., Wolman, I., Har-Toov, J., Amster, R., Peyser, M.R., 1996. Changes in uterine artery resistance to blood flow during puerperium – a longitudinal study. J. Matern. Fetal Investig. 6, 27–30.
Kirkinen, P., Dudenhausen, J., Baumann, H., Huch, A., Huch, R., 1988. Postpartum blood flow velocity waveforms of the uterine arteries. J. Reprod. Med. 33, 745–748.
Köster, K., Poulsen Nautrup, C., Günzel-Apel, A.R., 2001. A Doppler ultrasonographic study of cyclic changes of ovarian perfusion in the Beagle bitch. Reproduction 122, 453–461.
Krueger, L., Koerte, J., Tsousis, G., Herzog, K., Flachowsky, G., Bollwein, H., 2009. Transrectal Doppler sonography of uterine blood flow during the first 12 weeks after parturition in healthy dairy cows. Anim. Reprod. Sci. 114, 23–31.
Mortensen, C., Kelley, D., Warren, L., 2011. Supplemental l-arginine shortens gestation length and increases mare uterine blood flow before and after parturition. J. Equine Vet. Sci. 31, 514–520.
Mulic-Lutvica, A., Bakos, O., Bekuretzion, M., Axelsson, O., 2001. Ultrasonic evaluation of the uterus and uterine cavity after normal, vaginal delivery. Ultrasound Obstet. Gynecol. 18, 491–498.
Mulic-Lutvica, A., Eurenius, K., Axelsson, O., 2007. Longitudinal study of Doppler flow resistance indices of the uterine arteries after normal vaginal delivery. Acta Obstet. Gynecol. Scand. 86, 1207–1214.
Mulic-Lutvica, A., Eurenius, K., Axelsson, O., 2009. Uterine artery Doppler ultrasound in postpartum women with retained placental tissue. Acta Obstet. Gynecol. Scand. 88, 724–728.

Nakai, Y., Imanaka, M., Nishio, J., Maeda, T., Ozaki, A., Sun, T.T., Ogita, S., 1997. Uterine blood flow velocity waveforms during early postpartum course following caesarean section. Eur. J. Obstet. Gynecol. Reprod. Biol. 74, 121–124.

Nautrup, C.P., 1998. Doppler ultrasonography of canine maternal and fetal arteries during normal gestation. J. Reprod. Fertil. 112, 301–314.

Orfanou, D.C., Ververidis, H.N., Pourlis, A., Fragkou, I.A., Kokoli, A.N., Boscos, C.M., Taitzoglou, I.A., Tzora, A., Nerou, C.M., Athanasiou, L., Fthenakis, G.C., 2009. Post-partum involution of the canine uterus – Gross anatomical and histological features. Reprod. Domest. Anim. 44, 152–155.

Orfanou, D.C., Ververidis, H.N., Pourlis, A., Fragkou, I.A., Kokoli, A.N., Boscos, C.M., Taitzoglou, I.A., Tzora, A., Nerou, C.M., Athanasiou, L., Fthenakis, G.C., 2008. Experimental study of the post-partum involuting genital tract of the bitch. Part I. Clinical bacteriological and cytological features. Proceedings of the 6th International Symposium of Canine and Feline Reproduction, 175–176.

Pharr, J.W., Post, K., 1992. Ultrasonography and radiography of the canine postpartum uterus. Vet. Radiol. Ultrasound 33, 35–40.

Polisca, A., Scotti, L., Orlandi, R., Brecchia, Boiti, C., 2010. Doppler evaluation of maternal and fetal vessels during normal gestation in rabbits. Theriogenology 73, 358–366.

Scotti, L., Di Salvo, P., Bocci, F., Pieramati, C., Polisca, A., 2008. Doppler evaluation of maternal and foetal vessels during normal gestation in queen. Theriogenology 69, 1111–1119.

Tekay, A., Jouppila, P., 1993. A longitudinal Doppler ultrasonographic assessment of the alterations in peripheral vascular resistance of uterine arteries and ultrasonographic findings of the involuting uterus during the puerperium. Am. J. Obstet. Gynecol. 168, 190–198.

van der Heijden, O.W., Essers, Y.P., Fazzi, G., Peeters, L.L., Mey, J.G., van Eys, G.J., 2005. Uterine artery remodeling and reproductive performance are impaired in endothelial nitric oxide synthase deficient mice. Biol. Reprod. 72, 1161–1168.

van der Heijden, O.W., Essers, Y.P., Wijnands, E., Mey, J.G., Peeters, L.L., van Eys, G.J., 2009. Postpartum reversal of the pregnancy-induced uterine artery remodeling in young, aging, and eNOS-deficient mice. Reprod. Sci. 16, 642–649.

Yeager, A.E., Concannon, P.W., 1990. Serial ultrasonographic appearance of postpartum uterine involution in beagle dogs. Theriogenology 34, 523–525.