

Doppler ultrasonographic assessment of maternal and fetal arteries during normal feline gestation



P.G. Blanco ^{a,*}, R. Rodríguez ^a, S. Olguín ^a, A. Rube ^a, M. Tórtora ^a, C. Gobello ^b

^a Cardiology Service, 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

ARTICLE INFO

Article history:

Received 8 November 2013

Received in revised form 27 January 2014

Accepted 2 February 2014

Available online 15 February 2014

Keywords:

Doppler

Ultrasound

Uterine artery

Umbilical artery

Pregnancy

Cat

ABSTRACT

The aim of this study was to describe Doppler parameters of uterine, umbilical, fetal abdominal aorta, fetal renal and fetal internal carotid arteries, as well as fetal heart rate (FHR), during normal feline gestation. Fifteen, 1–4 years of age, weighing 2.5–3.9 kg, domestic short-hair pregnant queens, which were born in our institutional cat colony were included in this study. Color and pulsed-wave Doppler evaluations of uterine arteries were performed every 10 days (Day 0, 10, 20, 30, 40, 50, 60) from mating. Fetal Doppler and M-mode ultrasonography were performed to assess umbilical, fetal abdominal aorta, fetal renal, fetal internal carotid arteries and FHR. Both peak systolic velocity (PSV) and end diastolic velocity (EDV) of uterine artery increased up to parturition ($P < 0.01$), while resistance index (RI) decreased from Day 10 onwards ($P < 0.01$). From Day 40 onwards, RI of umbilical artery diminished, while PSV and EDV augmented ($P < 0.01$). Fetal abdominal aorta ($P < 0.01$), renal ($P < 0.01$) and internal carotid ($P < 0.01$) arteries diminished their RI from Days 40, 60 and 40 onwards, respectively. Both PSV and EDV of these three arteries increased progressively. Fetal heart rate was first registered on Day 20 when it began to increase up to Day 40 and then diminished to the end of gestation ($P < 0.01$). It is concluded that blood flow of uterine, umbilical, fetal abdominal aorta, fetal renal and fetal internal carotid arteries progressively increased during normal feline pregnancy, while FHR rose to mid gestation and then decreased up to parturition.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Doppler ultrasound is a non invasive useful tool to evaluate gestation in many mammalian species. This technique permits the examination of placental and fetal vessels providing information of fetoplacental health. As gestation progresses, the increasing density of fetal membranes and placental capillaries induces a rising blood flow of

the involved vessels (Papageorghiou et al., 2004; Reynolds et al., 2006).

Doppler ultrasound measures absolute velocities within an artery, from which resistance index (RI) is calculated. This index decreases during normal gestation in uterine, umbilical and other fetal arteries in many mammalian species (Bucca et al., 2005; Blanco et al., 2008).

In human and canine pregnancy, it has been reported that the absence of RI decrease of uterine, umbilical and fetal renal arteries is associated with adverse outcome (Coleman et al., 2000; Acharya et al., 2005; Blanco et al., 2011). For this reason, in pregnant women, Doppler has become a routine technique for identifying fetal compromise associated with an abnormal uteroplacental or fetal circulation.

* Corresponding author. Tel.: +54 221 423 6663/+54 221 479 0544; fax: +54 221 427 7378.

E-mail addresses: pgblanco@fcv.unlp.edu.ar, pgblanco@gmail.com (P.G. Blanco).

In the queen, Doppler reports in obstetrics are controversial. While Scotti et al. (2008) found a decrease of RI of uteroplacental arteries along with gestation, Pereira et al. (2012a) stated that the index remained unchanged. Furthermore, in one report, the RI of uterine arteries was found to peak between Days 33 and 43 and then diminished up to Day 48 (Brito et al., 2010), and to progressively decrease in the uterine horn containing the lower number of fetuses in another study (Pereira et al., 2012b).

Concerning umbilical artery velocimetry, Scotti et al. (2008) described that its RI diminished progressively during the second half of gestation. Conversely, Brito et al. (2010) stated that this index only decreased in two periods: between Days 22 and 40 and between Days 41 and 50 of gestation.

Fetal aorta was described during the last 5 weeks of feline pregnancy presenting a decrease of its RI (Scotti et al., 2008), and there is no information about this vessel during early gestation. In human fetuses, oligohydramnios with prolonged hypoxia is probably a consequence of decreased renal perfusion, evidenced by Doppler assessment of fetal renal artery (Mari et al., 1993). Additionally, RI of the fetal internal carotid artery is predictive for the development of an abnormal fetal heart rate trace (Groenenberg et al., 1993). In pregnant cats there are no reports of fetal renal and carotid arteries.

Fetal heart rate (FHR) can be first ultrasonographically detected between Days 16 and 17 of feline gestation (Zambelli and Prati, 2006), and it has been reported to remain unchanged in the course of this period (Verstegen et al., 1993). Considering that this parameter is widely used to monitor fetal health (Lopate, 2008; Traas, 2008; White, 2012), it is also interesting to determine eventual gestational variations as have been found in other species (Verstegen et al., 1993; Breukelman et al., 2006; Nagel et al., 2011). Physiological changes of Doppler parameters and FHR during feline gestation should be known so that unfavorable fetal conditions could be recognized.

To unveil the previously described controversies, the aim of this study was to describe Doppler parameters of uterine artery, umbilical artery, fetal abdominal aorta, fetal renal and fetal internal carotid arteries, as well as FHR, during normal feline gestation.

2. Materials and methods

2.1. Animals

Fifteen, 1–4 year-old, weighing 2.5–3.9 kg, domestic short-hair pregnant queens, which were born in our Institutional cat colony were included in this study. The animals were exposed to a 10 h dark, 14 h light photoperiod, fed with dry commercial premium cat food and water *ad libitum*.

Estrus cycle was monitored three times a week, based on behavior and vaginal cytology (Johnston et al., 2001). Queens were mated on the third or fourth day of estrus (Day 0) by fertile male cats. Considering all the animals, the whole period of mating was 1 month. This study was reviewed and approved by the Animal Care and Use Committee of the Veterinary School of the National University

of La Plata, Argentine and all experiments were conducted under the guidelines established in The Guide for The Care and Use of laboratory Animals, USA.

2.2. Study design and ultrasonographic evaluations

Pregnancy was confirmed in all the cases using two-dimensional ultrasonographic examination (Toshiba Core Vision Pro, Japan) with an 8-MHz linear-array transducer (England, 1998). Color and pulsed-wave Doppler evaluations of uterine arteries were performed every 10 days from mating day in all the queens, i.e. Days 0, 10, 20, 30, 40, 50, 60. The female cats were positioned in lateral recumbency without sedation and the hair of the ventral skin was clipped after 5 min of acclimatization. Acoustic gel was applied to the transducer and coupled directly to the skin. Two-dimensional ultrasonography was used to identify the uterine body in a transversal axis. Uterine arteries were localized at both sides of the uterine body with color flow mapping and pulsed-wave Doppler was used to obtain the waveforms (Alvarez-Clau and Liste, 2005).

Fetal Doppler and M-mode ultrasonography were performed in the most caudal fetus of the right uterine horn, according to previous reports (Di Salvo et al., 2006; Scotti et al., 2008). Umbilical artery, fetal abdominal aorta, fetal renal artery and fetal internal carotid artery were assessed by color and pulsed-wave Doppler from their first possible detection up to parturition (Scotti et al., 2008). To perform Doppler ultrasonography of the fetal renal artery, a longitudinal view of the fetal kidney was obtained. The renal artery was subsequently detected in the renal hilum of the fetus (Blanco et al., 2011). The fetal internal carotid artery was detected at each side of the deep portion of diencephalo-telencephalic vesicle (Beccaglia et al., 2008; Blanco et al., 2011). To minimize variability, three uniform consecutive waveforms were recorded by a trained operator. Measurements having an angle of insonation >20° were disregarded. Peak systolic velocity (PSV) and end diastolic velocity (EDV) were measured. Resistance index [(PSV–EDV)/PSV] was automatically calculated in all vessels (Dickey, 1997). Fetal heart rate was obtained with M-mode ultrasound as previously described (Verstegen et al., 1993). In all the queens, ultrasonographic evaluations were performed in periods no longer than 30 min.

2.3. Statistical analysis

Peak systolic velocity, EDV and RI of the left and right uterine arteries were compared using a Student's *t*-tests. Repeated measures ANOVA followed by Tukey test was carried out to evaluate the effect of time on PSV, EDV and RI of each artery and FHR, using litter size as a covariate (SPSS 18.0; SPSS, Chicago, IL, USA). The level of significance was set on $P < 0.05$.

3. Results

Parturition occurred normally 65 ± 1.6 (mean \pm SEM) days from mating in all the females. Litter size ranged from 2 to 5 healthy kittens, without having an effect on the analyzed parameters ($P > 0.1$).

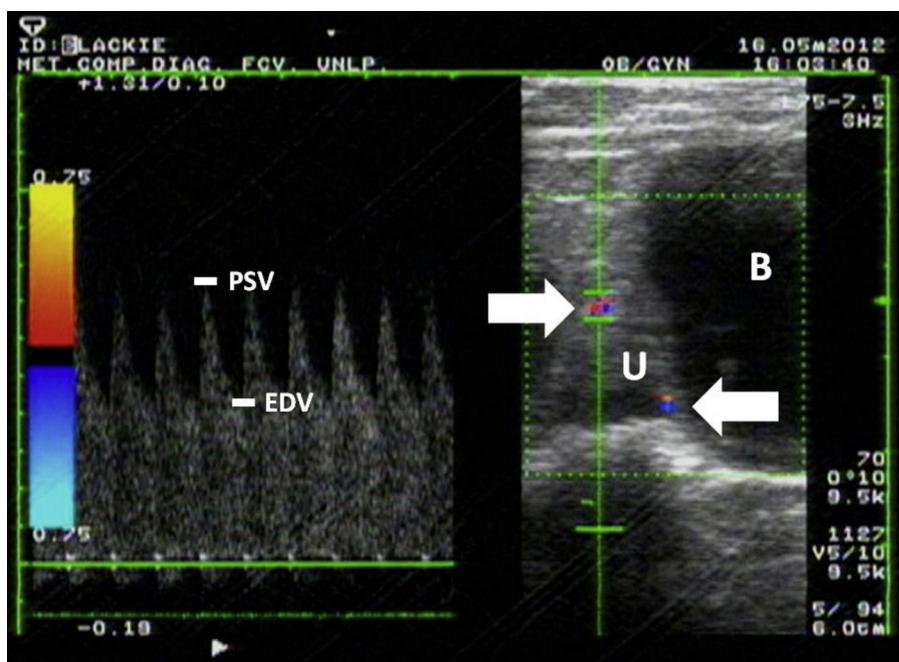


Fig. 1. Pulsed wave Doppler ultrasound register of left and right uterine arteries (white arrows) at each side of the uterine body (U), in a selected pregnant queen 50 days after mating. Bladder (B); peak systolic velocity (PSV); end diastolic velocity (EDV).

Original recordings of pulsed-wave Doppler ultrasound of uterine artery are shown in Fig. 1. No differences were found between left and right uterine arteries ($P>0.1$), therefore, values of PSV, EDV and RI were averaged. Uterine artery waveforms were characterized by the presence of end diastolic flow from Day 0 to the end of gestation. Both PSV and EDV of this artery increased up to parturition ($P<0.01$; Table 1). Conversely, RI of uterine artery progressively decreased in the course of the study from Day 10 onwards ($P<0.01$). On that Day, this parameter was 0.56 ± 0.01 , and on Day 60 it was 0.32 ± 0.00 (Fig. 2).

Umbilical artery was first detected on Day 20 of gestation. From Day 40 onwards, RI of this artery diminished ($P<0.01$; Fig. 3), while PSV and EDV augmented ($P<0.01$; Table 1). On that Day, RI showed a value of 0.96 ± 0.01 , and on Day 60 it fell to 0.72 ± 0.02 . At the time point of detection, this artery had only a systolic component of the waveform. On Day 30, a diastolic component was inconsistently observed, although it was constant from Day 40 to parturition.

Fetal abdominal aorta and fetal internal carotid artery were first observed on Day 20 and 30 of pregnancy, respectively, while fetal renal artery was first detected on Day

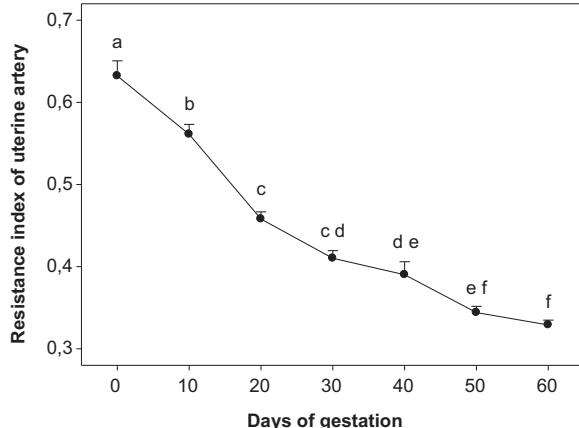


Fig. 2. Resistance index (mean \pm SEM) of uterine artery of 15 queens assessed every 10 days during normal gestation (Day 0=day of mating). Different letters indicate differences ($P<0.01$) between days.

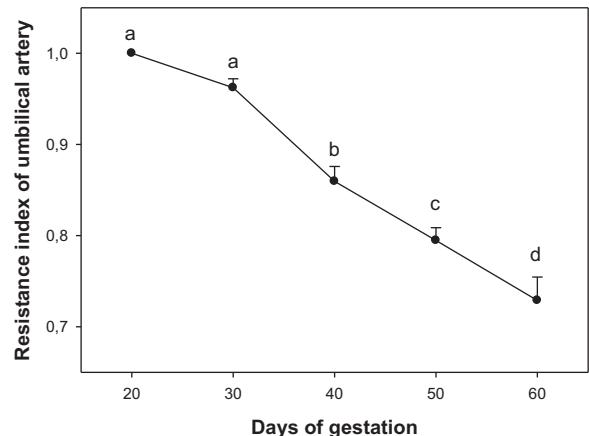


Fig. 3. Resistance index (mean \pm SEM) of umbilical artery of the same animals of Fig. 2. Different letters indicate differences ($P<0.01$) between days.

Table 1

Peak systolic velocity (PSV; mean \pm SEM) and end diastolic velocity (EDV; mean \pm SEM) of uterine artery (UA), umbilical artery (UMB), fetal abdominal aorta (Ao), fetal renal (FR) and fetal internal carotid (FIC) arteries of fifteen queens assessed every 10 days during normal gestation (Day 0 = day of mating).

| Artery | Days of gestation | | | | | | | |
|--------|-------------------|------------------------------|-------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|------------------------------|
| | 0 | 10 | 20 | 30 | 40 | 50 | 60 | |
| UA | PSV | 0.21 \pm 0.01 ^a | 0.21 \pm 0.00 ^a | 0.28 \pm 0.00 ^a | 0.45 \pm 0.03 ^b | 0.49 \pm 0.02 ^b | 0.57 \pm 0.06 ^{b,c} | 0.60 \pm 0.05 ^c |
| | EDV | 0.08 \pm 0.0 ^a | 0.10 \pm 0.0 ^{a,b} | 0.19 \pm 0.02 ^{b,c} | 0.28 \pm 0.02 ^{c,d} | 0.29 \pm 0.02 ^{c,d} | 0.37 \pm 0.04 ^d | 0.35 \pm 0.03 ^d |
| UMB | PSV | – | – | 0.04 \pm 0.00 ^a | 0.13 \pm 0.00 ^b | 0.31 \pm 0.03 ^c | 0.40 \pm 0.02 ^d | 0.41 \pm 0.01 ^d |
| | EDV | – | – | 0 \pm 0 ^a | 0.004 \pm 0.00 ^a | 0.04 \pm 0.00 ^b | 0.09 \pm 0.00 ^c | 0.11 \pm 0.01 ^c |
| Ao | PSV | – | – | 0.06 \pm 0.00 ^a | 0.19 \pm 0.01 ^b | 0.28 \pm 0.03 ^b | 0.41 \pm 0.03 ^c | 0.39 \pm 0.02 ^c |
| | EDV | – | – | 0 \pm 0 ^a | 0.003 \pm 0.00 ^a | 0.03 \pm 0.00 ^b | 0.06 \pm 0.00 ^c | 0.08 \pm 0.00 ^c |
| FR | PSV | – | – | – | – | 0.06 \pm 0.00 ^a | 0.07 \pm 0.00 ^a | 0.1 \pm 0.00 ^b |
| | EDV | – | – | – | – | 0.007 \pm 0.00 ^a | 0.01 \pm 0.00 ^b | 0.02 \pm 0.00 ^c |
| FIC | PSV | – | – | – | 0.05 \pm 0.00 ^a | 0.07 \pm 0.00 ^{a,b} | 0.08 \pm 0.00 ^b | 0.09 \pm 0.00 ^b |
| | EDV | – | – | – | 0.005 \pm 0.00 ^a | 0.01 \pm 0.00 ^{a,b} | 0.02 \pm 0.00 ^{b,c} | 0.03 \pm 0.00 ^c |

Means with different superscript in the same line indicate differences ($P < 0.01$) between days.

40. Fetal abdominal aorta ($P < 0.01$; Fig. 4), renal ($P < 0.01$; Fig. 5) and internal carotid ($P < 0.01$; Fig. 6) arteries diminished their RI from Days 40, 60 and 40, respectively. Both PSV and EDV of these three arteries increased progressively (Table 1). At the time point of first detection, fetal

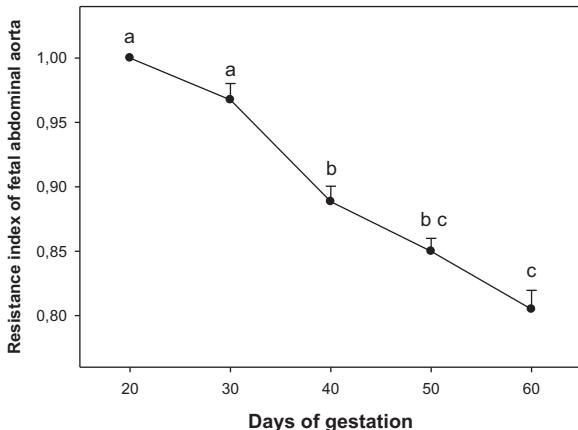


Fig. 4. Resistance index (mean \pm SEM) of fetal abdominal aorta of the same animals of Fig. 2. Different letters indicate differences ($P < 0.01$) between days.

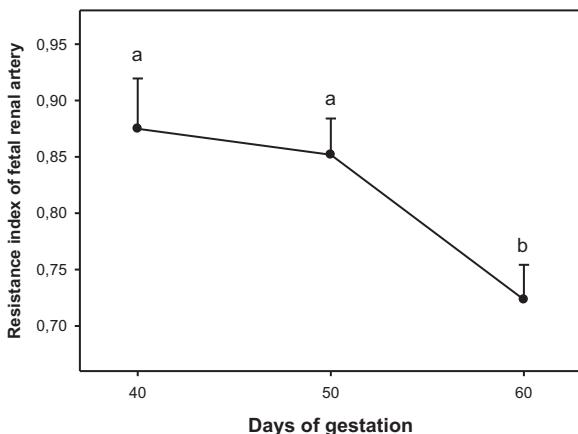


Fig. 5. Resistance index (mean \pm SEM) of fetal renal artery of the same animals of Fig. 2. Different letters indicate differences ($P < 0.01$) between days.

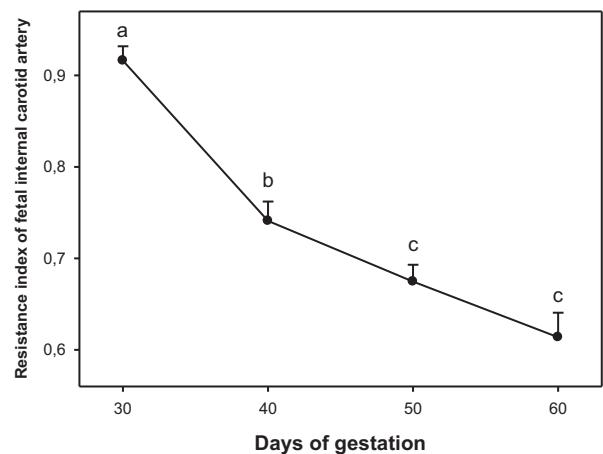


Fig. 6. Resistance index (mean \pm SEM) of fetal internal carotid artery of the same animals of Fig. 2. Different letters indicate differences ($P < 0.01$) between days.

abdominal aorta and internal carotid artery had RI values of 1.0 ± 0 and 0.91 ± 0.01 , respectively, and reached values of 0.80 ± 0.01 and 0.61 ± 0.02 on Day 60. Fetal renal artery showed a RI of 0.87 ± 0.04 on Day 40 and it decreased to 0.72 ± 0.03 on Day 60. Fetal heart rate was first registered on Day 20 with a value of 227 ± 3 bpm. Then, it began to increase up to Day 40 reaching 224 ± 1 bpm. Finally, it diminished to the end of gestation with a value of 223 ± 7 bpm on Day 60 ($P < 0.01$; Fig. 7).

4. Discussion

In the course of this study, maternal and fetal arteries blood flow progressively increased to parturition. No decrease in blood flow was detected in the studied vessels as previously found in other studies (Brito et al., 2010; Pereira et al., 2012a,b). Moreover, FHR also showed changes during pregnancy. This is consistent with what has been described for other mammalian species such as cows, horses, humans and dogs for normal gestation (Dickey, 1997; Bollwein et al., 2002, 2004; Blanco et al., 2011).

In this experiment, uterine artery presented a constant end diastolic flow (i.e. it was neither absent nor reversed), while PSV and EDV increased and RI decreased. This is

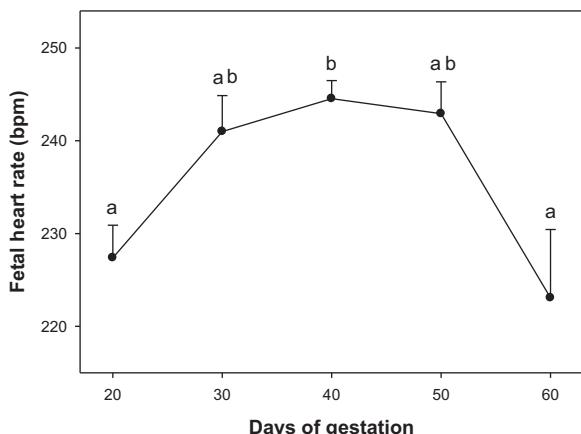


Fig. 7. Fetal heart rate (mean \pm SEM) of the same animals of Fig. 2. Different letters indicate differences ($P < 0.01$) between days.

in line with what has been described for uteroplacental arteries in pregnant queens (Scotti et al., 2008) and in disagreement with other reports for uteroplacental (Pereira et al., 2012a) and uterine arteries in this species (Brito et al., 2010; Pereira et al., 2012b). The increase in the uterine blood flow found in this study might be due to the increasing feline placental functional activity and fetal growth (Miglino et al., 2006). In addition, it has been suggested that uterine artery waveform is also a reflection of estrogen, progesterone and androgens effects, maternal vascular and endothelial function (Everett and Lees, 2012). It is worth noting that the absence of a decreasing rate of the uterine artery RI has been associated to adverse pregnancy outcome in humans and dogs (Cnossen et al., 2008; Blanco et al., 2011).

Absolute values of uterine artery RI found in this study were lower than those previously reported for pregnant cats (Brito et al., 2010; Pereira et al., 2012b). They were also lower than those described for canine pregnancy (Miranda and Domingues, 2010). Despite both species develop an endotheliochorial placenta (Walter and Schönkypl, 2006), this vascular difference might be due to some histological characteristics of feline placenta which has not been described in dogs (Dall'Aglio et al., 2012a,b). Each area in the zonary girdle of the feline placenta is supplied by a centrally located stem-artery. The periendothelial region between the maternal endothelium of this stem vessel and the surrounding syncytiotrophoblast is comparable to the human perivascular stem villous sheath (Walter and Schönkypl, 2006). Interestingly, in the course of human gestation, RI of uterine artery has similar values than those found here for feline gestation (Kurmanavicius et al., 1997; Bahlmann et al., 2012). This might be due to the previously described placental histological similarities.

Values of uterine artery PSV and EDV found in this study were higher than those previously reported in uterine as well as uteroplacental arteries in pregnant cats (Scotti et al., 2008; Pereira et al., 2012a). This might be due to the reduced angle of insonation used in this study. When the angle between the ultrasound beam and the blood flow is close to zero degrees, a value close to the real value of

the blood velocity is obtained. Importantly, when angle correction is used on very small or very tortuous vessels, such as the feline uterine arteries, it is easy to erroneously increase or decrease the actual angle of insonation and, thereby, to obtain an incorrect estimation of the velocity. As the angle increases, the blood velocity is progressively underestimated (Detti et al., 2010).

Resistance index of uterine artery diminished in these cats from Day 10 onwards. The beginning of this decrease occurred earlier than in other species (Alvarez-Clau and Liste, 2005; Blanco et al., 2011). Considering that in queens, bidimensional ultrasound can be used to diagnose gestation from Day 11 and embryo observation is possible from Day 16 onwards (Zambelli and Prati, 2006; Davidson and Baker, 2009), Doppler ultrasound would be an early tool for feline pregnancy diagnosis. In this aspect, further studies in early pseudopregnant cats would be necessary to unveil whether uterine artery RI decrease is pregnancy specific or not.

As expected, umbilical artery blood flow augmented throughout feline gestation. It was measured from Day 20 onwards, as previously reported for this species (Pereira et al., 2012a). On that day, the yolk sac is completely vascularized by blood vessels originated from the umbilical cord (Miglino et al., 2006). Furthermore, in this study EDV of this artery was first detected on Day 30, which is consistent with a previous report in cats (Scotti et al., 2008). Resistance index of umbilical artery decreased in the course of pregnancy, as previously described in this species (Scotti et al., 2008). This might be due to the branches of the blood vessels of the umbilical cord that begin to appear between Day 25 and 30 of gestation, supplying both the yolk sac and the placental girdle (Miglino et al., 2006).

Fetal abdominal aorta increased its blood flow throughout gestation as previously described in pregnant cats (Scotti et al., 2008; Pereira et al., 2012a). This artery was detected on Day 20, 6 days later than in other study (Pereira et al., 2012a). This is probably due to that in that report, fetal aorta was measured at the level of its arch, while in this study the abdominal portion was assessed adding information about the peripheral circulation to the hind limbs, spleen, skeletal muscles and mesentery (Akalin-Sel and Campbell, 1992).

To our knowledge, this is the first report of Doppler velocimetry of fetal renal and internal carotid arteries in the course of feline pregnancy. Blood flow increased in both vessels during gestation as previously reported in humans and dogs (Mari et al., 1993; Morales-Roselló, 2002; Blanco et al., 2011). Fetal renal artery was first registered on Day 40 and its RI diminished on Day 60, which is consistent with what has been found in canine species (Blanco et al., 2011). This fall might be due to the late appearance of the cortical region, which can be ultrasonographically detected from Day 50 onwards in feline fetal kidneys (Zambelli and Prati, 2006). The development of glomerular tissue might be responsible for the augmented blood flow in the fetal renal artery. Fetal internal carotid artery was first observed on Day 30 of gestation. In this study, the deep portion of diencephalo-telencephalic vesicle was observed in the fetal skull from Day 30, as previously described in cats (Beccaglia et al., 2008). For this reason, it was possible to detect the

internal carotid artery at each side of this structure, earlier than in dogs (Blanco et al., 2011). Resistance index of this vessel decreased during the present study. This is probably due to the development of the fetal encephalic structures (Morales-Roselló, 2002).

Feline FHR increased initially from Day 20 up to Day 40 and then described a decrease up to parturition. This result agrees with previous reports for canine FHR, which presented low values by the time of detection, rose to mid gestation and then diminished up to parturition (Verstegen et al., 1993). However it is not in line with previous reports in cats, in which FHR was reported to remain stable during pregnancy (Verstegen et al., 1993). The data obtained in the present study is useful to determine the values of FHR that should be considered as indicative of fetal stress.

In this study, Doppler ultrasound evidenced the increasing blood flow in the course of normal feline gestation. Further work is needed to compare normal and complicated pregnancy values and then to find out if these parameters could predict abnormal feline gestation. Although, series of examinations would be needed to detect changes in the normal trend of Doppler parameters, a single determination with aberrant values could be indicative of obstetrical disease in this species.

5. Conclusions

It is concluded that blood flow of uterine artery, umbilical artery, fetal abdominal aorta, fetal renal and fetal internal carotid arteries progressively increased during normal feline pregnancy, while FHR rose to mid gestation and then decreased up to parturition.

Conflict of interest statement

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

Acknowledgments

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

References

- Acharya, G., Wilsgaard, T., Berntsen, G.K., Maltau, J.M., Kiserud, T., 2005. Reference ranges for serial measurements of umbilical artery Doppler indices in the second half of pregnancy. *Am. J. Obstet. Gynecol.* 192, 937–944.
- Akalin-Sel, T., Campbell, S., 1992. Understanding the pathophysiology of intra-uterine growth retardation: the role of the 'lower limb reflex' in redistribution of blood flow. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 23, 79–86.
- Alvarez-Clau, A., Liste, F., 2005. Ultrasonographic characterization of the uterine artery in the nonestrus bitch. *Ultrasound Med. Biol.* 31, 1583–1587.
- Bahlmann, F., Fittschen, M., Reinhard, I., Wellek, S., Steiner, E., 2012. Reference values for blood flow velocity in the uterine artery in normal pregnancies from 18 weeks to 42 weeks of gestation calculated by automatic Doppler waveform analysis. *Ultraschall Med.* 33, 258–264.
- Beccaglia, M., Anastasi, P., Grimaldi, E., Rota, A., Faustini, M., Luvoni, G.C., 2008. Accuracy of the prediction of parturition date through ultrasonographic measurement of fetal parameters in the queen. *Vet. Res. Commun.* 32, S99–S101.
- 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., Tórtora, M., Díaz, J.D., Gobello, C., 2011. Doppler ultrasonographic assessment of maternal and fetal blood flow in abnormal canine pregnancy. *Anim. Reprod. Sci.* 126, 130–135.
- Bollwein, H., Baumgartner, U., Stolla, R., 2002. Transrectal Doppler sonography of uterine blood flow in cows during pregnancy. *Theriogenology* 57, 2053–2061.
- Bollwein, H., Weber, F., Woschée, I., Stolla, R., 2004. Transrectal Doppler sonography of uterine and umbilical blood flow during pregnancy in mares. *Theriogenology* 61, 499–509.
- Breukelman, S., Mulder, E.J., van Oord, R., Jonker, H., van der Weijden, B.C., Taverne, M.A., 2006. Continuous fetal heart rate monitoring during late gestation in cattle by means of Doppler ultrasonography: reference values obtained by computer-assisted analysis. *Theriogenology* 65, 486–498.
- Brito, A.B., Miranda, S.A., Ruas, M.R., Santos, R.R., Domingues, S.F., 2010. Assessment of feline fetal viability by conceptus echobiometry and triplex Doppler ultrasonography of uterine and umbilical arteries. *Anim. Reprod. Sci.* 122, 276–281.
- Bucca, S., Fogarty, U., Collins, A., Small, V., 2005. Assessment of fetoplacental well-being in the mare from mid-gestation to term: transrectal and transabdominal ultrasonographic features. *Theriogenology* 64, 542–557.
- Crossen, J.S., Morris, R.K., ter Riet, G., Mol, B.W., van der Post, J.A., Coomarasamy, A., Zwinderman, A.H., Robson, S.C., Bindels, P.J., Kleijnen, J., Khan, K.S., 2008. Use of uterine artery Doppler ultrasonography to predict pre-eclampsia and intrauterine growth restriction: a systematic review and bivariable meta-analysis. *Can. Med. Assoc. J.* 178, 701–711.
- Coleman, M.A., McCowan, L.M., North, R.A., 2000. Mid-trimester uterine artery Doppler screening as a predictor of adverse pregnancy outcome in high-risk women. *Ultrasound Obstet. Gynecol.* 15, 7–12.
- Dall'Aglio, C., Polisca, A., Boiti, C., Ceccarelli, P., 2012a. Immunolocalization of leptin and its receptor in the placenta of cats. *Acta Histochem.* 114, 719–722.
- Dall'Aglio, C., Pascucci, L., Mercati, F., Polisca, A., Ceccarelli, P., Boiti, C., 2012b. Immunohistochemical detection of the orexin system in the placenta of cats. *Res. Vet. Sci.* 92, 362–365.
- Davidson, A.P., Baker, T.W., 2009. Reproductive ultrasound of the bitch and queen. *Top. Companion Anim. Med.* 24, 55–63.
- Detti, L., Akiyama, M., Mari, G., 2010. Doppler blood flow in obstetrics. *Curr. Opin. Obstet. Gynecol.* 14, 587–593.
- Di Salvo, P., Bocci, F., Zelli, R., Polisca, A., 2006. Doppler evaluation of maternal and fetal vessels during normal gestation in the bitch. *Res. Vet. Sci.* 81, 382–388.
- Dickey, R., 1997. Doppler and ultrasound investigation of uterine and ovarian blood flow in infertility and early pregnancy. *Hum. Reprod.* 3, 467–503.
- England, G.C.W., 1998. Ultrasonographic assessment of abnormal pregnancy. *Ultrasonography* 28, 849–867.
- Everett, T.R., Lees, C.C., 2012. Beyond the placental bed: placental and systemic determinants of the uterine artery Doppler waveform. *Placenta* 33, 893–901.
- Groenenberg, I.A., Hop, W.C., Bogers, J.W., Santema, J.G., Vladimiroff, J.W., 1993. The predictive value of Doppler flow velocity waveforms in the development of abnormal fetal heart rate traces in intrauterine growth retardation: a longitudinal study. *Early Hum. Dev.* 32, 151–159.
- Johnston, S.D., Root-Kustritz, M.V., Olson, P.N., 2001. The feline estrus cycle. In: *Canine and Feline Theriogenology*. BW Saunders, Philadelphia, PA, pp. 396–405.
- Kurmanavicius, J., Florio, I., Wisser, J., Hebsch, G., Zimmermann, R., Müller, R., Huch, R., Huch, A., 1997. Reference resistance indices of the umbilical, fetal middle cerebral and uterine arteries at 24–42 weeks of gestation. *Ultrasound Obstet. Gynecol.* 10, 112–120.
- Lopate, C., 2008. Estimation of gestational age and assessment of canine fetal maturation using radiology and ultrasonography: a review. *Theriogenology* 70, 397–402.
- Mari, G., Kirshon, B., Abuhamad, A., 1993. Fetal renal artery flow velocity waveforms in normal pregnancies and pregnancies complicated by polyhydramnios and oligohydramnios. *Obstet. Gynecol.* 81, 560–564.

- Miglino, M.A., Ambrósio, C.E., dos Santos Martins, D., Wenceslau, C.V., Pfarer, C., Leiser, R., 2006. *The carnivore pregnancy: the development of the embryo and fetal membranes*. Theriogenology 66, 1699–1702.
- Miranda, S.A., Domingues, S.F., 2010. Conceptus ecobiometry and triplex Doppler ultrasonography of uterine and umbilical arteries for assessment of fetal viability in dogs. Theriogenology 74, 608–617.
- Morales-Roselló, J., 2002. Doppler sonography of normal fetal vertebral and internal carotid arteries during pregnancy. J. Clin. Ultrasound 30, 257–263.
- Nagel, C., Aurich, J., Aurich, C., 2011. Heart rate and heart rate variability in the pregnant mare and its foetus. Reprod. Domest. Anim. 46, 990–993.
- Papageorgiou, A.T., Yu, C.K.H., Nicolaides, K.H., 2004. The role of uterine artery Doppler in predicting adverse pregnancy outcome. Best Pract. Res. Clin. Obstet. Gynaecol. 18, 383–396.
- Pereira, B.S., Pinto, J.N., Freire, L.M., Campello, C.C., Domingues, S.F., da Silva, L.D., 2012a. Study of the development of uteroplacental and fetal feline circulation by triplex Doppler. Theriogenology 77, 989–997.
- Pereira, B.S., Freire, L.M., Pinto, J.N., Domingues, S.F., Silva, L.D., 2012b. Triplex Doppler evaluation of uterine arteries in cyclic and pregnant domestic cats. Anim. Reprod. Sci. 130, 99–104.
- Reynolds, L.P., Caton, J.S., Redmer, D.A., Grazul-Bilska, A.T., Vonnahme, K.A., Borowicz, P.P., Luther, J.S., Wallace, J.M., Wu, G., Spencer, T.E., 2006. Evidence for altered placental blood flow and vascularity in compromised pregnancies. J. Physiol. 572, 51–58.
- 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.
- Traas, A.M., 2008. Resuscitation of canine and feline neonates. Theriogenology 70, 343–348.
- Verstegen, J.P., Silva, L.D., Onclin, K., Donnay, I., 1993. Echocardiographic study of heart rate in dog and cat fetuses in utero. J. Reprod. Fertil., Suppl. 47, 175–180.
- Walter, I., Schönkyl, S., 2006. Extracellular matrix components and matrix degrading enzymes in the feline placenta during gestation. Placenta 27, 291–306.
- White, S.C., 2012. Prevention of fetal suffering during ovariohysterectomy of pregnant animals. J. Am. Vet. Med. Assoc. 240, 1160–1163.
- Zambelli, D., Prati, F., 2006. Ultrasonography for pregnancy diagnosis and evaluation in queens. Theriogenology 66, 135–144.