GENERAL INTRODUCTION

The clinical examination of the mother is important to assess the progress of pregnancy, but it is not sufficient to get information on the fetal well-being.

When the maternal conditions are compromised, the health of fetuses could also be affected; on the other hand, a good health status of the mother does not guarantee that the fetuses are all healthy.

In cases of systemic involvement, as well as for changes in the maternal homeostasis due to infectious agents, toxic molecules, or hormones and drugs that may reach embryos or fetuses by transplacental passage, fetal survival could be negatively affected. During the early stages of gestation embryo resorptions are often asymptomatic and later, in case of fetal death, bitches may not show clinical symptoms (Johnston, 1983; England et al., 1990; Yeager et al., 1992; Kutzler et al., 2003; Root-Kustritz, 2005; Kim et al., 2007; Davidson, 2008; Sridevi, 2013). For all these reasons, the fetal well-being, i.e. appropriate fetal development according to gestational age and fetal health, has to be assessed by specific diagnostic tools.

In human medicine until the 50s, obstetricians put primary emphasis on the maternal survival and health. The health of the fetus has gained increasing attention only after 1960 thanks to the technological support that allowed the direct assessment of the physical fetal conditions "in utero" (Patrick, 1989).

In veterinary medicine during the last decades, the ultrasound examination of the reproductive system in the bitch has been deeply investigated. Many authors have described specific ultrasonographic findings of the canine pregnancy and nowadays ultrasonography represents the gold standard method for the evaluation of a pregnant bitch (Johnston, 1983; Poffenbarger & Feeney, 1986; England et al., 1990; Yeager et al., 1992; Kutzler et al., 2003; Root-Kustritz, 2005; Kim et al., 2007; Davidson, 2008; Lopate, 2008; Michel et al., 2011; Sridevi, 2013). It represents the most safe and sensitive method for the pregnancy diagnosis from the day 25 of gestation (Concannon et al., 1989; England et al., 1990; Yeager et al., 1992; Miles, 1995). In addition, this technique allows to monitor fetal viability and development, to recognize the stage of pregnancy, and to estimate the date of delivery (Johnston, 1983; Poffenbarger & Feeney, 1986; England et al., 1990; Yeager et al., 1992; Kutzler et al., 2003; Root-Kustritz, 2005; Kim et al., 2007; Davidson, 2008; Lopate, 2008; Michel et al., 2011; Sridevi, 2013).

Ultrasonographic evaluation of the fetal development

Many authors have examined the timing of the first ultrasonographic appearance of certain extra-fetal and fetal structures during the canine pregnancy (Table 1).

Table 1. *The gestational age at first appearance of ultrasonographic features during canine pregnancy (based on England et al., 1990; Yeager et al., 1992; Boroffka, 2005; Kim & Son, 2007).*

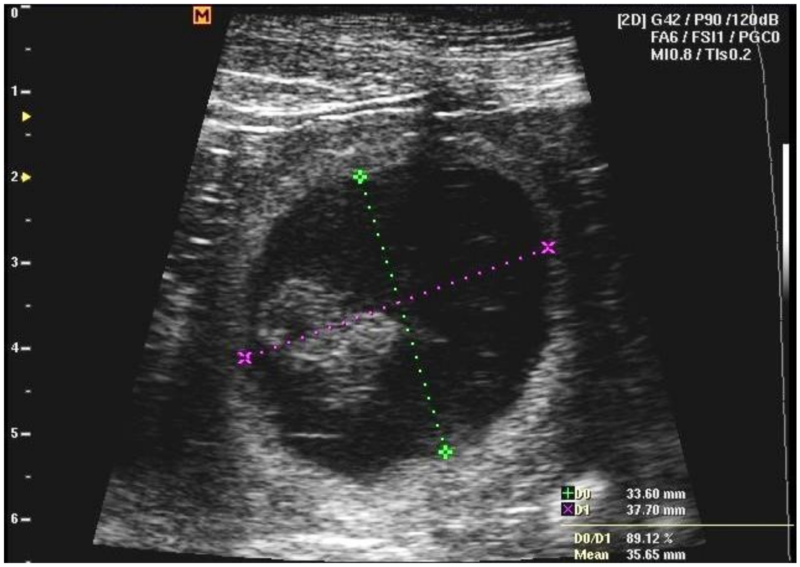
Features identified	**Gestational Age (days after ovulation)**
Conceptus as a 1 - 2 mm uterine vesicle	19-20
Presence of the embryonic mass	22-23
Heartbeat	23-24
Yolk sac membrane	25-27
Bipolar embryo shape	26-28
Allantoic membrane	27-31
Limb bud	27-31
Placenta develops zonary shape	27-29
Head	27-30
Chorionic cavity exceeds the size of the yolk sac	28-30
Anechoic area in head	29-33
Stomach	29-33
Dorsal tubular spinal column	30-36
Urinary bladder	31-35
Collapsing of the elongated yolk sac	31-34
Fetal movement	32-34
Axial skeleton	33-34
Lung hyperechoic vs liver	34-36
Liver hypoechoic vs abdomen	35-38
Trunk diameter exceeds diameter of head	38-40
Trunk diameter exceeds 50% of chorionic cavity diameter	38-42
Crown-rump length exceeds length of placenta	38-42
Kidneys	40-46
Eyes	40-46
4 Cardiac chambers	40-44
Trunk diameter exceeds 50% of uterine outside diameter	46-48
Intestines	58-62

These information are useful to detect whether the pregnancies progress regularly (Concannon, 2000; Nyland & Matton 2002). However, the estimation of gestational age based on the assessment of organ development is unpractical because it would require a daily ultrasonographic assessment of embryos/fetuses. Moreover, the first identification of specific anatomical features depends by the examiner experience and by the resolution of the used equipment (Lenard et al., 2007). To overcome these limits, the gestational age is commonly estimated by the ultrasonographic measurements of extra-fetal and fetal structures.

Different extra-fetal parameters can be evaluated during early pregnancy. From day 20 up to approximately day 40 of gestation, the chorionic cavity (Figure 1) has a typical spherical appearance with well-defined margins and anechoic content. The internal diameters (inner chorionic cavity, ICC), made at 90° angles from one side of the trophoblastic decidual reaction to the other, can be easily recognized and measured (England et al., 1990; Yeager et al., 1992; Luvoni & Grioni, 2000; Kutzler et al., 2003; Luvoni & Beccaglia, 2006).

In the same gestational period, the outer uterine diameter (OUD) at the implantation sites, the placental thickness (PT) and length (PL) can also be estimated (England et al., 1990; Yeager et al., 1992; Luvoni & Grioni, 2000; Son et al. 2001). However, the ICC measurement is easier to obtain than the OUD, PT and PL because the uterine wall and the annexes have less defined margins than ICC (England et al., 1990; Yeager, 1992; Luvoni & Grioni, 2000; Son et al. 2001; Kutzler et al., 2003; Michel et al., 2011).

Figure 1. *Ultrasonographic measurement of inner chorionic cavity (ICC) in a bitch (36 days before parturition).*



During fetal growth, different biometric parameters can be evaluated (Lopate, 2008; Michel et al., 2011). In the longitudinal plane, the fetal length (crown-rump length, CRL), between the most rostral point and the caudal edge of the fetus, and, in the transverse plane, the body diameter (BD) at the level of the stomach and liver, can be measured.

When the fetal head can be distinguished from the body, fetal head diameter is measured as its largest cross-sectional diameter. When parietal bones are identified in the coronal section of the head, the biparietal diameter (BP, Figure 2) is the parameter of choice. The deep portion of diencephalo-telencephalic vesicle (DPTV) can also be visualized in the same scan. The BP is the distance between the two parietal bones of the skull, and the DPTV, is an ovoid anechoic structure with well-defined margins symmetrical to the longitudinal fissure separating the two cerebral hemispheres (Figure 2).

The major and minor axis of the fetal heart (diameter of fetal heart, HDF) have also been considered as biometrical parameters and used to evaluate the gestational age (England et al., 1990; Yeager, 1992; Moriyoshi et al. 1996; Luvoni & Grioni, 2000; Son et al. 2001; Kutzler et al., 2003; Michel et al., 2011).

During each examination, it is advisable to obtain the average of at least two measurements of the same extra-fetal or fetal parameter in different fetuses, to minimize the risk of inconsistent measurements. In the case of singleton pregnancy, different parameters should be assessed on the same fetus (Luvoni & Beccaglia 2006), although this could be also helpful in non-singleton pregnancies (Lopate, 2008; Michel et al., 2011).

Figure 2. *Ultrasonographic measurement of biparietal diameter (BP) and deep portion of diencephalo-telencephalic vesicle (DPTV) in a bitch (20 days before parturition).*

The table 2 summarizes the extra-fetal and fetal ultrasonographic parameters and the related formulae for the gestational age and/or days before parturition in the bitch.

Table 2. *Ultrasonographic parameters for the evaluation of fetal development in the bitch.*

Biometric parameters	Equations	Breed/Size	References
Inner Chorionic Cavity (ICC)	GA = 19.66 + (6.27 * cm)	Beagle	Yeager et al., 1992;
	DBP = (mm * 82.13) / 1.8	Medium	Luvoni & Grioni, 2000;
	DBP = (mm * 68.88) / 1.53	Small	Luvoni & Grioni, 2000;
	DBP = 63.2 * (18.58 + 0.71 * mm)	Maltese	Son et al., 2001
	DBP = 63.4 *(18.92 + 0.65 * mm)	Yorkshire	Son et al., 2001
	GA = (6 * cm) + 20	Medium	Nyland & Matton, 2002
	DBP = (mm * 74.68) / 1.75	Yorkshire	Socha & Janowski, 2011
	DBP = (mm * 84.66) / 1.86	Golden	Socha & Janowski, 2011
	DBP = 44.76 – (4.34 * cm)	German Shepherd	Groppetti et al., 2015a
Outer Uterine Diameter (OUD)	GA = 17.39 + 4.98 * cm	Beagle	Yeager et al., 1992;
	DBP = (mm - 80.78) / 1.57	Medium	Luvoni & Grioni, 2000;
	DBP = (mm - 85.71) / 1.83	Small	Luvoni & Grioni, 2000;
Placental Thickness (PT)	DBP = (mm - 18.99) / 0.45	Medium	Luvoni & Grioni, 2000
	DBP = (mm - 5.8) / 0.12	Small	Luvoni & Grioni, 2000
Crown-Rump Length (CRL)	GA = 24.64 + 4.54 * cm - 0.24 * cm^2	Beagle	Yeager et al., 1992;
	GA = (3 * cm) + 27	Medium	Nyland & Matton, 2002
Body Diameter (BD)	GA = 22.89 + 12.75 * cm - 1.17 * cm^2	Beagle	Yeager et al., 1992;
	GA = (7 * cm) + 29	Medium	Nyland & Matton, 2002
	Mm = 9.738 - 0.6575 * GA + 0.02558 * GA^2	Beagle	Moriyoshi et al., 1996
Placental Length (PL)	GA= 18.74 + 3.58 * cm	Beagle	Yeager et al., 1992;
Head Diameter (HD)	GA = 21.08 + 14.88 * cm – 0.11 * cm^2	Beagle	Yeager et al., 1992;
	DBP = 63.2 * (24.7 + 1.54 * mm)	Maltese	Son et al., 2001
	DBP = 63.4 *(23.89 + 1.63 * mm)	Yorkshire	Son et al., 2001
	Mm = 23.18 + 1.098 * GA + 0.00016 * GA^2	Beagle	Moriyoshi et al., 1996
	GA = (15 * cm) + 20	Medium	Nyland & Matton, 2002
Biparietal Diameter (BP)	DBP = (mm - 29.18) / 0.7	Medium	Luvoni & Grioni, 2000
	DBP = (mm - 25.11) / 0.61	Small	Luvoni & Grioni, 2000
	DBP = (mm – 24.5) / 0.62	Yorkshire	Socha & Janowski, 2011
	DBP = (mm - 31.19) / 0.8	Golden	Socha & Janowski, 2011
	DBP = 38.65 – (12.86 * cm)	German Shepherd	Groppetti et al., 2015a
Body Diameter (BD) & Head Diameter (HD)	GA = 6 * cm(HD) + 3 * cm(BD) + 30	Retriever	Nyland & Matton, 2002
Body Diameter (BD) & Biparietal Diameter (BP)	DBP = 34.27 - 5.89 * cm(BP) -2.77 * cm(BD)	Retriever	England et al., 1990
Deep Portion of Diencephalo-Telencephalic Vesicle (DPTV)	DBP = (mm - 10.11) / 0.24	Small	Beccaglia & Luvoni, 2004
	DBP = (mm - 14.15) / 0.4	Medium	Beccaglia & Luvoni, 2004
	DBP = (mm - 10.27) / 0.24	Large	Beccaglia et al., 2008
Diameter of Fetal Heart (HDF)	Mm = -9,1221 + 0.2297 * GA + 0.0057 * GA^2	Beagle	Moriyoshi et al., 1996

GA: gestational age; DBP: days before parturition

The accuracy of this estimation has been studied by different authors (Luvoni & Grioni, 2000; Kutzler et al., 2003; Beccaglia & Luvoni 2006; Lenard et al., 2007; Lopate, 2008; Michel et al., 2011; Socha & Janowski, 2014).

The gestational period in which the examination is performed mainly affects the accuracy of parameters. that resulted higher during early pregnancy, than afterwards (Yeager et al., 1992; Son et al., 2001; Kutzler et al., 2003; Beccaglia & Luvoni, 2006; Socha et al., 2012; Socha & Janowski, 2014). According to Kutzler et al., 2003, the most accurate predictions of parturition date are obtained when ICC measurements are performed on day 30. The accuracy of BP, that is measured for an extended period (from week 5 to 9) is highly consistent during the 6th week of gestation (Beccaglia & Luvoni, 2012).

It has been demonstrated that the fetal sex ratio and the litter size do not generally influence the accuracy of parturition date prediction (Kutzler et a., 2003; Beccaglia & Luvoni, 2006), but the BP accuracy is higher in normal litter size than in small and large litters (Beccaglia & Luvoni, 2006; Groppetti et al., 2015a). This may be due to the fact that BP measurement might be affected by individual variability of growth when few fetuses are present, or it may be less accurate when the overlapping of multiple fetuses in the same ultrasonographic image field occurs (Beccaglia & Luvoni, 2006).

In dogs, the wide breed variability requires specific reference curves of biometric values based on different breed sizes (Luvoni & Grioni, 2000; Son et al., 2001; Kutzler et al., 2003; Luvoni & Beccaglia, 2006; Socha et al., 2012). Some authors demonstrated that maternal bodyweight affects the accuracy of parturition date prediction (Kutzler et al., 2003), but ICC and BP are both highly reliable when size-related specific formulae are applied (Beccaglia & Luvoni, 2006; Socha et al., 2015). Thus, an equally accurate prediction can be obtained both in early and late gestation (Beccaglia & Luvoni, 2006).

The fetal development has been deeply investigated in small and medium dogs, whereas only few information are available for large and giant size bitches even though they are very well represented in the one hundred most popular canine breeds (Sverdrup Borge et al., 2011; Tønnessen et al., 2012). For these dogs, specific formulae for the evaluation of fetal growth are not yet available. Kutzler and colleagues (2003) suggested the use of a correction factor to adjust the difference between actual and predicted parturition date obtained with previously published equations for dogs of smaller size (England et al., 1990; Yeager et al., 1992). Although the formulae for medium dogs (Luvoni & Grioni, 2000) have been also used in large and giant breeds (Socha & Janowski, 2014; Socha et al., 2015), specific equations for these dogs would allow the most accurate prediction of parturition term (Michel et al. 2011; Socha & Janowski, 2014; Socha et al., 2015).

Evaluation of fetal health

Ultrasonography

To assess the fetal health, different fetal and the extra-fetal parameters can be evaluated during pregnancy by the B-mode ultrasonography and the Echo Color Doppler examination.

The elective method to assess embryo/fetal viability or to recognize the interruption of pregnancy, at any time and at any stage of the development of the conceptuses, is the ultrasonography. In dogs, the embryonic death before the day 35 after ovulation (followed by the complete resorption of the conceptus) is not recognizable radiologically. With the ultrasound exam, this event is characterized by a decrease in the volume of the embryo vesicle, an increase echogenicity of the fluid, an absence of heartbeat, and a distortion and collapse of the embryonic mass (Konde, 1988; Concannon, 2003). The uterine wall appears moderately hyperechoic and a small amount of free fluid can be found into the lumen of the organ (Concannon, 2003).

After the day 35 of pregnancy, fetal death may be associated with a vaginal discharge, but it can also go unnoticed. In all cases, the ultrasound examination detects changes in the fluids and the absence of the heartbeat. When an abortion occurs, after the expulsion of the died puppies, the uterus will show the typical appearance of post-partum (Concannon, 2003). Only in case of late fetal death, X-rays can identify skeletal deformities, altered relationships between different skeletal sites, the cranial bones spaced and the presence of gas around fetal bodies (Rendano, 1983; Toal et al., 1986; Miles, 1995; Lopate, 2008; Lamm & Makloski, 2012).

B-mode ultrasonographic exam

The B-mode ultrasound exam provides many important information about fetal well-being through the analysis of some indicators as the fetal development, the amount and the echogenicity of fetal fluids, the placental thickness (PT), and the fetal movements. When one or more of these indicators do not fall within the normal range, the suffering condition of the fetus is usually defined as "fetal stress" (Table 3).

Table 3. *Parameters to identify fetal stress in the bitch by B-mode ultrasonographic exam.*

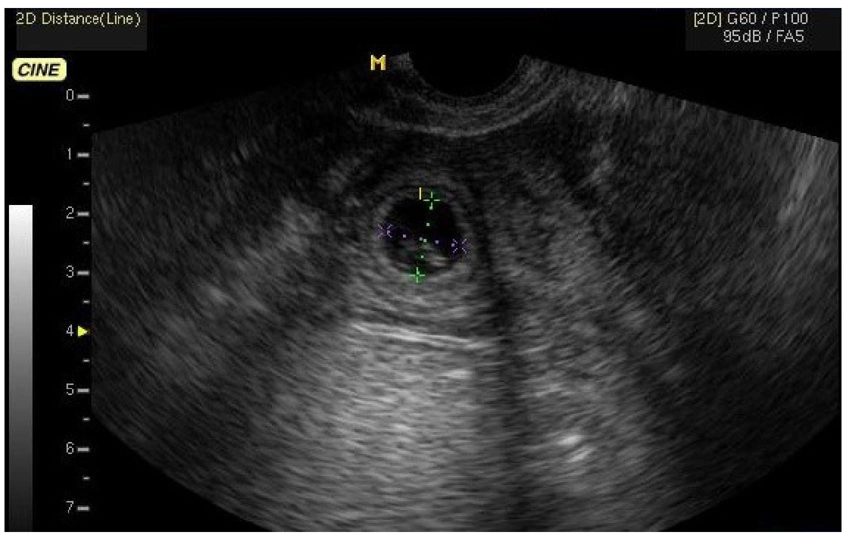
Parameters	**References**
Fetal development	See previous paragraph
Abdominal:Biparietal diameter ratio	Zone & Wanke, 2001
Placental thickness	Lopate, 2008
Fetal fluids	Zone & Wanke, 2001; Lopate, 2008
Fetal movements	Yeager et al., 1992; Bocking et al., 1985; Davidson, 2001; Zone & Wanke, 2001; England & Russo, 2006

The parameters to evaluate the fetal development in dogs have been extensively discussed in the previous paragraph (see Tables 1 and 2).

Some authors suggested that an intrauterine growth retardation may be suspected when the ratio between abdominal and biparietal diameters is less than 2 from day 48 of gestation (Zone & Wanke, 2001). Puppies with a low ratio are at risk for early neonatal loss, since the low ratio is usually associated with a 20% decrease of bodyweight at birth (Zone & Wanke, 2001).

In dogs, the placental thickness (Figure 3) must not exceed 1.2 cm along the whole pregnancy. Thickening or edema of the placenta indicate diminished ability of the organ to drain fetal waste fluids properly, alterations or abnormalities of blood flow, or placentitis (Lopate, 2008).

Figure 3. *Ultrasonographic measurement of placental thickness (yellow line).*



Along pregnancy, the amount of fetal fluid decreases as the fetus itself enlarges. Quantitative and qualitative assessment of fetal fluids can be performed by the ultrasonography. An increase or an abnormal decrease of these fluids may be due to abnormalities of placental functions with alteration of blood flow and/or decreased ability to drain the products of the fetal catabolism, or to rupture of the fetal membranes. A variation in the quality of the fluids, as an increased echogenicity can be determined by a hemorrhage with premature detachment of the placenta or to the passage of meconium (Zone & Wanke, 2001; Lopate, 2008). Fetuses generally do not survive for long time after the placenta begins to detach, although the detachment may be partial or complete (Lopate, 2008).

Several studies have analyzed and quantified the fetal movements in humans and livestock (Dawes et al., 1972; Ruckebusch, 1972; Patrick et al., 1982; Di Renzo et al., 1994a; Di Renzo et al., 1994b; Baska-Vincze et al., 2014), but little information are available in the dog (Davidson, 2001). The canine fetal movements are clearly visible by ultrasound from the day 35 of pregnancy and more frequent activities are the back bending of the head and the extension of the limbs (Yeager et al., 1992; Zone & Wanke, 2001; England & Russo, 2006). It has been suggested that the fetal movements are associated with an increased heart rate (Bocking et al., 1985; Davidson, 2001).

In human medicine breathing movements of the fetus can be recognized from the 10th week (De Vries et al., 1982), but in veterinary medicine, the dynamic of respiratory movements has been described only in the sheep (Dawes et al., 1972; Boddy et al., 1974; Patrick et al., 1987) and no information are available for dogs.

Echo Color Doppler exam

The purpose of the application of the Doppler in the obstetric monitoring of human and animal species is to evaluate the hemodynamic characteristics of the fetal-maternal circulation and to identify high-risk pregnancies (Fleischer & Emerson, 1994; Reed et al., 1996; Nautrup, 1998; Nicolaides et al., 2000; Bollwein et al., 2002; Dubiel et al., 2003; Bollwein et al., 2004; Di Salvo et al., 2006; Blanco et al., 2008).

The arterial blood of the mother, rich in oxygen and nutrients, through the placenta reaches the fetus via the umbilical vein. Some peculiarities, such as, the venous ductus, that connects the portal vein to the caudal vena cava, the ductus arteriosus, that combines the pulmonary trunk to the aorta, and the oval foramen (interatrial) characterize the fetal cardiovascular system that only at birth becomes as that of the adults (Götze, 1955). Furthermore, during pregnancy, the fetal lungs do not function and the pulmonary circulation is bypassed.

For the evaluation of fetal stress the parameters/structures described in the Table 4 are usually examined with the Doppler.

Table 4. *Parameters to identify fetal stress in the bitch by Echo Colour Doppler exam.*

Parameters	References
Utero-placental arteries	Nautrup, 1998; Di Salvo et al., 2006
Umbilical artery and vein	Nautrup, 1998; Di Salvo et al., 2006
Fetal aorta	Nautrup, 1998; Di Salvo et al., 2006
Fetal common carotid artery	Nautrup, 1998
Fetal caudal vena cava	Di Salvo et al., 2006
Fetal heart rate	Lopate, 2008

In the dog, the low resistance blood flow of the utero-placental arteries is characterized by a systolic peak, a small diastolic wave and by a relatively high speed of the end diastole (Nautrup, 1998; Di Salvo et al., 2006). Pulsatility and resistivity indexes are related to the growth of the placenta (Di Salvo et al., 2006).

In all species, at the umbilical level, the blood flows of the umbilical artery and vein are simultaneously present. In dogs, the umbilical cord can be identified from the week 4 of gestation with color Doppler and from the 5th week with two-dimensional ultrasonography. The blood flow in the umbilical artery is only systolic until the 6th week of pregnancy, and then the diastolic wave can also be identified. The umbilical vein is characterized by uniform flow with flat waves (Nautrup, 1998; Di Salvo et al., 2006).

The fetal aorta is characterized by high flow velocities recorded in two standard regions: in the thoracic area immediately above the diaphragm and in the abdominal region before the iliac arteries emerge. In dogs, no significant variation between the thoracic and abdominal region has been observed and the blood flow of the aorta until the 6th week of pregnancy is only systolic, whereas in the late phase of gestation the diastolic peak becomes evident (Nautrup, 1998; Di Salvo et al., 2006).

In canine fetuses, the common carotid artery can be recognized from the week 6 of gestation. This vessel is characterized by a quick systolic flow, with accelerations and decelerations, and by a diastolic flow with flat velocity (Nautrup, 1998). The flow in this vessel is pulsatile: a minor diastolic peak follows the systolic wave. Sometimes, a third retrograde wave due to atrial contraction can be observed (Maulik, 1999; Di Salvo et al., 2006).

Finally, in the bitch, the different degree of patient cooperation and the physiological respiratory arrhythmia, that determines a lengthening of the diastole, may influence parameters of the vessel flow (pulsatility index, resistivity index, ratio between the systolic and diastolic speed) (Nautrup, 1998). Moreover, the normal ranges of the obstetric Doppler parameters differ depending on the size of the dog (Blanco et al., 2008).

Fetal Heart Rate

Several authors have investigated maternal and fetal heart rate (MHR and FHR) features, their relationship and their mutual influence, to define clinical parameters of maternal and fetal well-being.

During pregnancy, the cardiovascular systems of the mother and the fetuses closely interact and the adaptation of the maternal system ensures a proper development of the fetus (Van Leeuwen et al., 2009).

Studies in pregnant women demonstrated an increase of blood volume that induces a chronic distention of the cardiac ventricles, followed by a positive inotropic effect and an increased activation of the sinoatrial node (Bader et al., 1955; Robson et al., 1989; Spatling et al., 1992; Khodiguian et al., 1996). The concurrent decrease in peripheral resistance ensures a proper spraying of the uterus to promote an adequate fetal development (Mone et al., 1996; Valensise et al., 2000).

Also in the pregnant bitch circulating blood volume and stroke volume increase (Brooks & Keil, 1994a; Brooks & Keil, 1994b; Johnston et al., 2001; Williams et al., 2007; Abbott, 2010; Blanco et al., 2011). In the late pregnancy, between 50 and 60 days, both the hypertrophy of the wall and the increase of the diameter of the left ventricle are well evident at the echocardiographic exam (Williams et al., 2007; Abbott, 2010; Blanco et al., 2011). The increased density of the fetal membranes and the increased number of placental capillaries induce a significant reduction in the resistance of the uterine artery resulting in a hypotension that provides an optimal blood supply to the fetus (Nautrup, 1998; Di Salvo et al., 2006; Blanco et al., 2009). Moreover, along pregnancy characterized by the predominance of the sympathetic tone, MHR and systolic function increase until delivery to support placental and fetal requirements (Williams et al., 2007; Abbott, 2010; Blanco et al., 2011). Therefore, the pregnancy induces some hemodynamic changes and the MHR tends to be higher to give adequate blood supply to the fetus (Lucio et al., 2009). However, several factors, regardless of the pregnant status, such as size, age, temperament, training etc., might influence the HR and therefore the MHR (Hamlin et al., 1967; Fleischer & Emerson, 1994; Bodey & Michell, 1996; Hezzell et al., 2013).

The FHR represents the most important parameter to estimate fetal health both in human and in veterinary medicine (Blanco et al., 2008; Gil et al., 2014). In the bitch, the embryonic heartbeat can be recognized by the ultrasound exam from the day 23 after the LH surge as a brilliant flicker in the embryo (Concannon et al., 1989; Yeager & Concannon, 1990; England et al., 1990; Yeager et al., 1992; Verstegen et al., 1993).

With the B-mode ultrasound exam the evaluation of the heart anatomy can be performed along fetal development. With the M-mode or Echo Color Doppler the cardiovascular function can be evaluated. It has been reported that a healthy and viable embryo/fetus shows a heart rate of 220-240 bpm (beats per minute). A value of FHR between 180 and 220 bpm is considered an early sign of fetal distress, whereas a frequency less than 140-160 bpm indicates a severe fetal stress usually due to hypoxia (Verstegen et al., 1993; Davidson, 1998; Zone & Wanke, 2001). Recently, some authors reported that acceleration and deceleration of FHR in all of the fetuses might be observed in the last hours of gestation and it could be considered a parameter to identify the approaching delivery (Gil et al., 2014).

In human medicine the FHR is generally detected by the cardiotocography (CTG) or the echo color Doppler, but the CTG, which simultaneously detects uterine activity, is considered the gold standard technique for the peri-partum monitoring (Fischer, 1979).

Some years ago, a cardiotocographic device for bitches (WhelpWise; Veterinary Perinatal Specialties Inc.; Wheat Ridge, Colorado) was introduced into the market. The instrument allows to correlate the FHR oscillations and the uterine contractions to identify uterine dysfunctions or fetal distress (Davidson & Eilts, 2006; Lopate, 2008). In women the application of this method is fully standardized and commonly used at the end of gestation, whereas in the bitch it raises some critical issues. In the woman the transducer is secured by a belt on the abdomen and it is precisely placed on the basis of fetal position. For the monitoring of twins it is equipped with two Doppler transducers to detect the two individual FHRs. The problem in the bitch is that a single transducer is placed in the lateral abdomen of small size dogs or in the back of those of greater size (Davidson & Eilts, 2006). The use of a single transducer in the presence of multiple fetuses implies that the recorded signals are disturbed by the interference and the overlapping of fetal heart beats. In addition, the positioning of the transducer based on the size of the mother, rather than on the position of the fetus, can affect the results. Therefore, the FHRs detected in the bitch cannot be considered as accurate as those obtained in the woman.

To obtain good Doppler signals and not to create artifacts, it is necessary to take into account several aspects such as the angles of incidence, the size and depth of the heart, and the fetal movements. For this reason, the echo color Doppler represents the more appropriate technique for the FHR evaluation.

In human medicine, some authors investigated the influence of the MHR on the trend of FHR. The studies in domestic animals are limited to the sheep in which, during the last quarter of gestation, the FHR follows the maternal circadian trend. Furthermore, the increase of the frequency of beats in the mother seems to coincide with fetal movements (Bocking et al., 1985).

The paucity of information regarding the mutual influence of MHR and FHR in canine pregnancy prompts to investigate their relationship. The FHR alone may not be sufficient for a reliable evaluation of fetal well-being and influencing factors have not yet been assessed.

AIMS

The main goal of a physical examination of a pregnant bitch is the evaluation of fetal development and fetal and maternal well-being.

As previously reported, the fetal development has been deeply investigated in small and medium dogs, whereas only few information are available for large and giant size.

Thus, the aim of this study was to derive the growth curves of ICC and BP in large and giant size bitches and to evaluate their accuracy. The effects of litter size and fetal sex ratio on the accuracy of the prediction were also investigated (**Paper 1**).

In addition, only few parameters are available to objectively assess the fetal health during canine pregnancy. Among them, the FHR is generally used, but the availability of reference values of the ratio FHR/MHR could better contribute to the evaluation of the fetal health at different gestational ages, than the single FHR values. For this purpose, the trend of FHR and FHR/MHR ratio in bitches of different pre-gestational bodyweight was evaluated during pregnancy (**Paper 2**).

MATERIALS & METHODS

Growth curves of ICC and BP in large and giant size bitches (**Paper 1**)

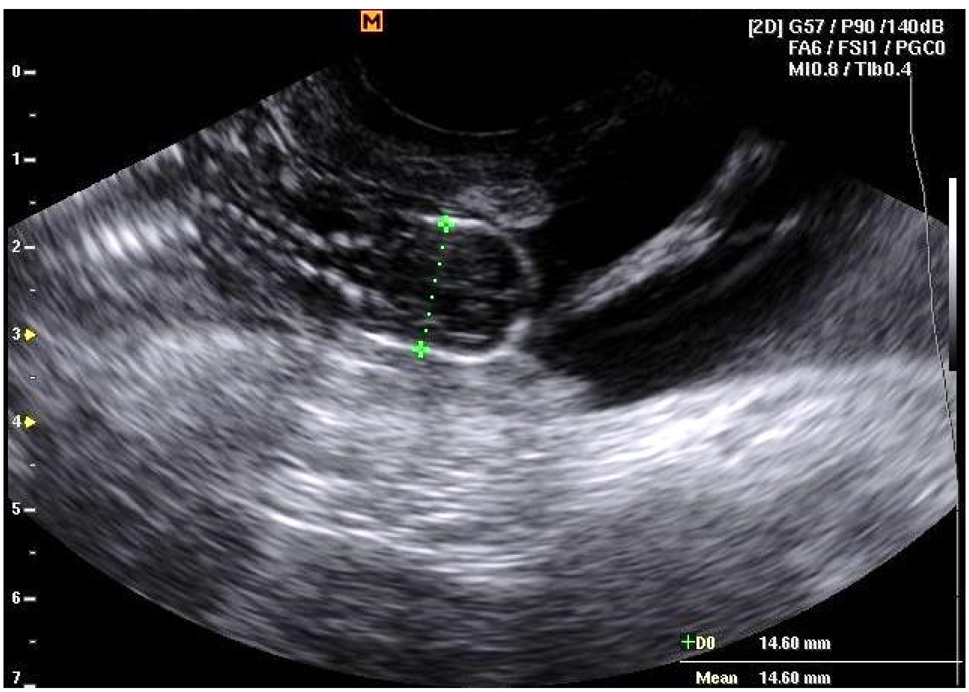
Eight large size (26-40 kg) bitches (Bergamasco Shepherd, Boxer, Doberman, German Shepherd, and Old English Shepherd) and 9 giant size (>40 kg) bitches (Great Dane, Bernese Mountain Dog, and Newfoundland), aged between 2 and 8 years presented to the Department for breeding management and pregnancy evaluation, were included in this study. Informed owner consent was obtained.

All bitches were healthy at the physical examination. For breeding management, the day of the ovulation was considered to be when plasma progesterone concentration ranged between 4-10 ng/ml (Arbeiter, 1993; Lévy & Fontbonne, 2007), as evaluated using an Enzyme Linked Fluorescent Assay (MiniVidas, BioMerieux, Marcy l'Etoile, France).

Serial ultrasonographic exams were performed weekly from day 20 after mating until parturition. Bitches were positioned in lateral recumbency, transmission gel was applied, and two-dimensional, gray-scale, real-time ultrasound images were produced using a 7.5 MHz microconvex probe (SonoAce 8800, Medison Co. Ltd., Seoul, Korea). During early pregnancy, inner diameter of chorionic cavity, and in late pregnancy the biparietal diameter (Figure 4) were measured. At least three measurements of ICC or BP, according to the gestation period, were recorded and the mean values were calculated. The time of actual parturition, the litter size, and the sex of the puppies were reported by the owners.

Statistical analysis: the relationship between ICC or BP growth and days before parturition was analyzed by a linear regression model. The growth equations for both parameters were derived as $y=a+bx$ (y=days before parturition, x=measurement in mm of ICC or BP, a=intercept coefficient and b=first order coefficient) and the regression coefficients were analyzed by the Student's T test ($p<0.05$, Software Stat Plus 2009).

Figure 4. *Ultrasonographic measurement of Biparietal Diameter (BP) in a bitch 20 days before parturition.*



Accuracy of ICC and BP for the prediction of parturition day in large and giant size bitches (**Paper 1**)

To assess the accuracy of the prediction, measurements of ICC and BP were performed in 65 and 102 ultrasound examinations of large size bitches and in 39 and 52 of giant size bitches with unknown breeding dates.

As previously reported, the prediction was considered accurate when the difference between actual and predicted parturition date was within ±1 day and ±2 days (Beccaglia & Luvoni, 2006).

To evaluate the effect of litter size on the accuracy, data were grouped for small (<5 pups), normal (5-9 pups) and large (>9 pups) litters in large and giant bitches (Beccaglia et al., 2008).

Moreover, predictions within ±1 day and ±2 days were analyzed on the basis of fetal sex ratio in terms of numerical prevalence (≥2) of one gender.

Statistical analysis: data were analyzed by Chi-Square test and the level of significance was set at $p<0.05$.

Assessment of trend of FHR and FHR/MHR ratio in bitches of different pre-gestational bodyweight during pregnancy (**Paper 2**)

Seventeen client-owned pregnant bitches of different breeds (Shih-tzu, Shetland, Jack Russell Terrier, Weimaraner, Boxer, and Great Dane), and pre-gestational bodyweights (5.8-68 kg) aged between 2 and 7 years presented to the Department for breeding management and pregnancy evaluation, were included in this study. Informed owner consent was obtained.

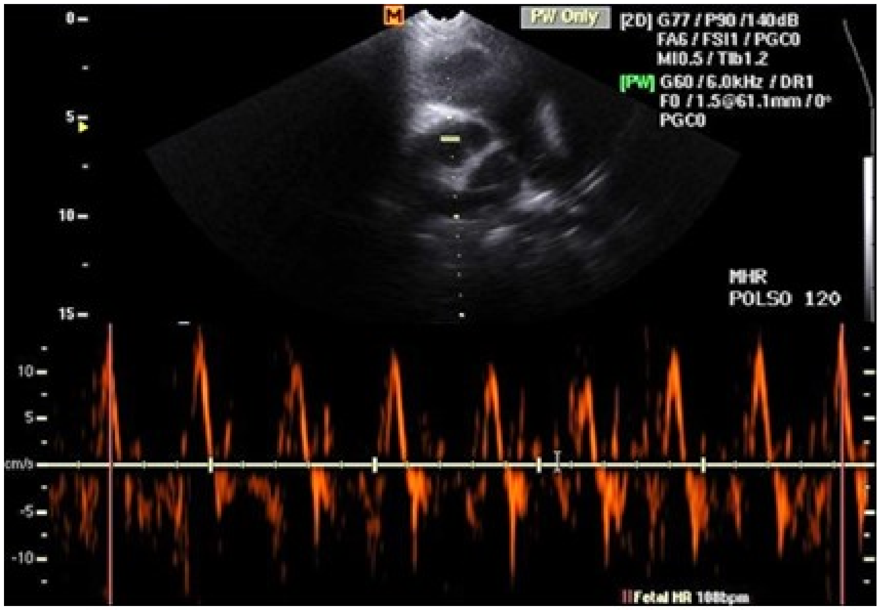
All bitches were healthy at the physical examination and with no history and signs of cardiac diseases. For breeding management, the day of the ovulation was considered when plasma progesterone concentrations ranged between 4-10 ng/ml (Arbeiter, 1993; Lévy & Fontbonne, 2007), as evaluated using an Enzyme Linked Fluorescent Assay (MiniVidas, BioMerieux, Marcy l'Etoile, France).

According to owner's availability, ultrasound examinations were performed in 5 bitches twice a week from day 21 after ovulation, and in 12 bitches at week 4, 7, and 9 of pregnancy.

Two-dimensional, gray-scale, real-time ultrasound and ecocolordoppler images were produced using a 7.5 MHz microconvex probe (SonoAce 8800, Medison Co. Ltd., Seoul, Korea).

The bitches were positioned in lateral recumbency, transmission gel was applied and MHR was evaluated at the level of the aortic valve for three times (at the beginning of the examination, after 10 minutes and at the end of the examination) to reduce and control the stress-effect induced by the restraint (Figure 5).

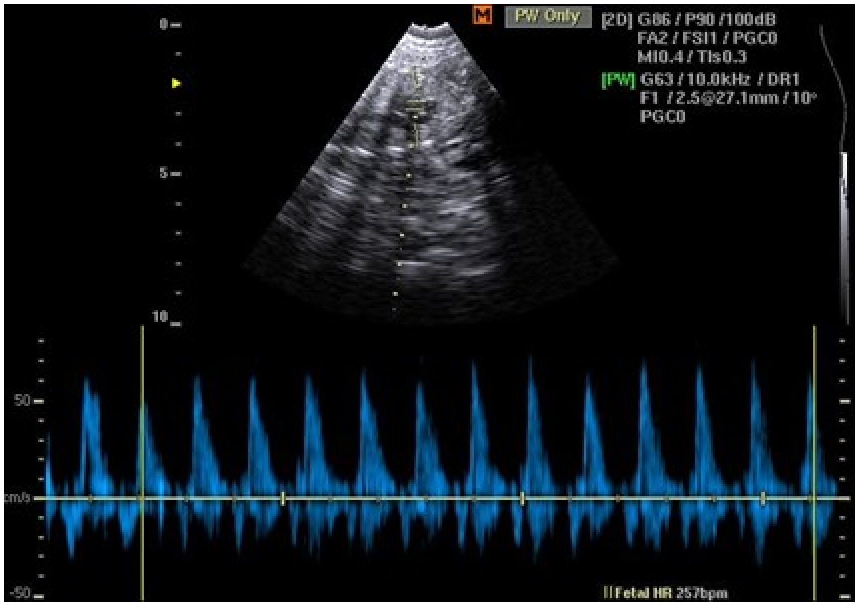
Figure 5. *Echo Color Doppler evaluation at the aortic level of the canine maternal heart rate (MHR).*



Fetuses' normal development was assessed by the measurement of ultrasonographic extra-fetal and fetal parameters (Luvoni & Grioni, 2000, Alonge et al., 2015). Fetal heart rates of at least three different fetuses (in litter size >3) were recorded in each examination (Figure 6).

The owners reported the day of parturition and the neonatal survival. Only data from uncomplicated pregnancies with no evidence of embryo, fetal or neonatal loss were included in the statistical analysis.

Figure 6. *Echo Color Doppler evaluation of the fetal heart rate (FHR) during canine pregnancy.*



Statistical analysis: a polynomial regression model was adopted to analyze the relationship between FHR, MHR, FHR/MHR ratio and independent variables (pre-gestational maternal bodyweight and gestational age, in terms of days from parturition). Statistical significance was set at $p \leq 0.05$ (Software Statistica 7 for Windows platform).

Growth curves of ICC and BP in large and giant size bitches (**Paper 1**)

The regression analysis resulted in a significant relationship between days before parturition and ICC or BP ($p<0.001$).

The derived equations for the prediction of parturition day in large and giant bitches are reported in table 5.

Table 5. *Growth curves of ICC and BP in large and giant size bitches*.

Parameter	**Large Bitches**		**Giant Bitches**	
	Equation	**R^2 Coefficient**	**Equation**	**R2 Coefficient**
ICC diameter	y = (x-105.1)/2.5	0.92	y = (x-88.1)/1.9	0.97
BP diameter	y = (x-88.1)/1.9	0.99	y = (x-29)/0.7	0.97

y=days before parturition, x=measurement in mm of ICC or BP,
a=intercept coefficient, b=first order coefficient.
ICC inner chorionic cavity; BP biparietal.

Accuracy of ICC and BP for the prediction of parturition day in large and giant size bitches (**Paper 1**)

The overall accuracy ±2 days of both parameters was significantly higher ($p<0.05$), than the accuracy ±1 day (Table 6). In giant bitches, the accuracy of ICC (±1 day and ±2 days) was significantly more accurate than that of BP.

Table 6. *Accuracy of parturition day prediction in large and giant size bitches.*

Bitch size	**±1 day**		**±2 days**	
	ICC diameter, n (%)	**BP diameter, n (%)**	**ICC diameter, n (%)**	**BP diameter, n (%)**
Large	42/65 (64.6)^{a}	64/102 (62.8)^{a}	54/65 (83.1)^{b}	90/102(88.3)^{b}
Giant	31/39 (79.5)^{A}	31/52 (59.6)^{B}	39/39 (100)^{C}	44/52 (84.6)^{D}
Overall	*73/104 (70.2)**	*95/154 (61.7)**	*93/104 (89.4)***	*134/154 (87)***

ICC inner chorionic cavity; BP biparietal.
Different superscripts denote significant differences within rows ($p<0.05$).

With regard to litter size, no differences ($p>0.05$) were observed in large bitches for both parameters (Table 7).

Table 7. *Accuracy of parturition day prediction based on litter size in large bitches.*

Litter size	**±1 day**		**±2 days**	
	ICC diameter, n (%)	**BP diameter, n (%)**	**ICC diameter, n (%)**	**BP diameter, n (%)**
Large (>9)	6/9 (66.7)	12/23(52.2)	9/9 (100)	20/23 (87)
Normal (5-9)	30/47 (63.8)	45/64 (70.3)	38/47 (80.9)	58/64 (90.6)
Small (<5)	6/9 (66.7)	7/15 (46.7)	7/9 (77.8)	12/15 (80)

ICC inner chorionic cavity, BP biparietal. No significant differences within columns.

In giant bitches, only the accuracy of the prediction by BP was significantly lower ($p<0.05$) in small, than normal litter size (Table 8).

Table 8. *Accuracy of parturition day prediction based on litter size in giant bitches.*

Litter size	**±1 day**		**±2 days**	
	ICC diameter, n (%)	**BP diameter, n (%)**	**ICC diameter, n (%)**	**BP diameter, n (%)**
Large (>9)	9/13 (69.2)	5/9 (55.6)a	13/13 (100)	7/9 (77.8)a
Normal (5-9)	10/12 (83.3)	22/28 (78.6)a	12/12 (100)	27/28 (96.43)a
Small (<5)	12/14 (85.7)	4/15 (26.7)b	14/14 (100)	9/15 (60)b

ICC inner chorionic cavity, BP biparietal.
Different superscripts denote significant differences within columns ($p<0.05$).

No effect of fetal sex ratio was observed on the accuracy (±1 day and ±2 days) of ICC and BP (Tables 9, 10).

Table 9. *Accuracy of parturition day prediction based on sex ratio in large bitches*.

Sex ratio	**±1 day**		**±2 days**	
	ICC diameter, n (%)	**BP diameter, n (%)**	**ICC diameter, n (%)**	**BP diameter, n (%)**
>Males	11/18 (61.1)	19/33 (57.6)	13/18 (72.2)	29/33 (87.9)
Males=Females	24/37 (64.9)	25/38 (65.7)	31/37 (83.8)	37/39 (94.9)
>Females	7/10 (70)	20/30 (66.7)	10/10 (100)	24/30 (77.8)

ICC Inner chorionic cavity, BP Biparietal. No significant differences within columns.

Table 10. *Accuracy of parturition day prediction based on sex ratio in giant bitches*.

Sex ratio	**±1 day**		**±2 days**	
	ICC diameter, n (%)	**BP diameter, n (%)**	**ICC diameter, n (%)**	**BP diameter, n (%)**
>Males	7/9 (77.8)	6/12 (50)	9/9 (100)	10/12 (83.3)
Males=Females	18/22 (81.8)	15/25 (60)	22/22 (100)	21/25 (84)
>Females	6/8 (75)	10/15 (66.7)	8/8 (100)	13/15 (86.7)

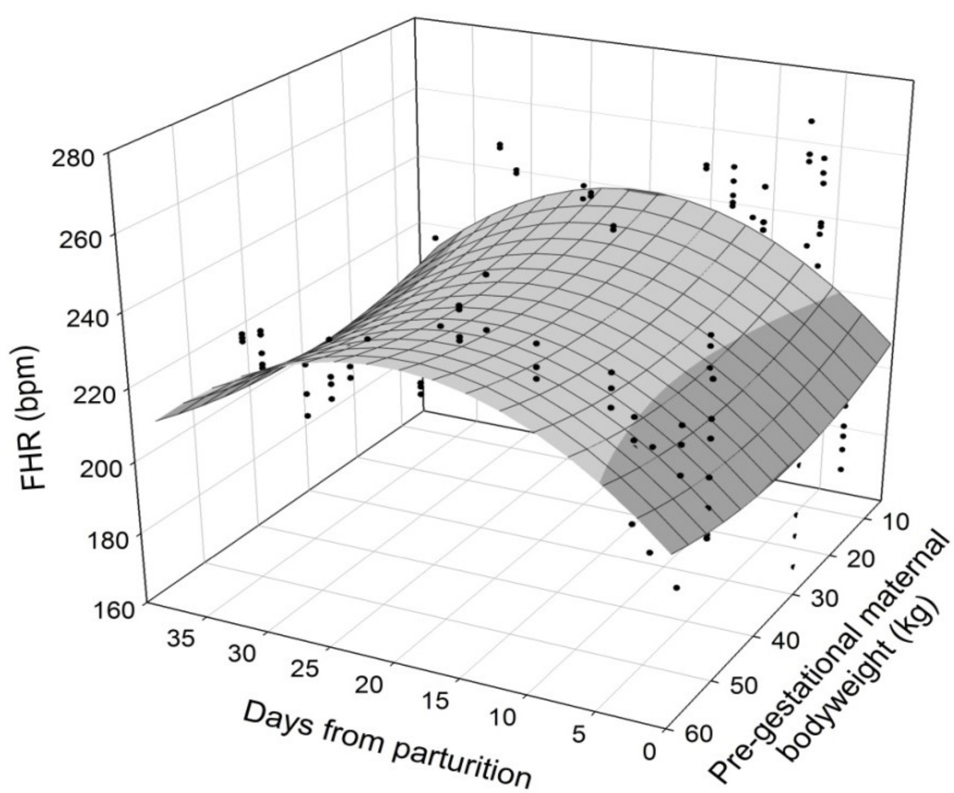
ICC Inner chorionic cavity, BP Biparietal. No significant differences within columns.

Assessment of trend of FHR and FHR/MHR ratio in bitches of different pre-gestational bodyweight during pregnancy (**Paper 2**)

Results include eleven uncomplicated pregnancies of bitches of different pre-gestational bodyweight (5.8-68 kg).

Fetal heart rates fitted a multiple quadratic regression (saddle, Fig. 7), with significance at $p \leq 0.02$ of all independent variables. Multiple r was about 0.50, a mean value, with a low determination coefficient ($r^2 = 0.25$).

Figure 7. *Quadratic regression for fetal heart rate (FHR) in bitches as a function of different pre-gestational bodyweight at different gestational age (days from parturition).*



The coefficients of the regression, the significance, and the confidence intervals are reported in Table 11. An increase of FHR was observed from 35 to 20 days before parturition. After the maximum, the curve followed a decreasing pattern until parturition. Higher values of FHR were observed in bitches of lowest and highest bodyweight.

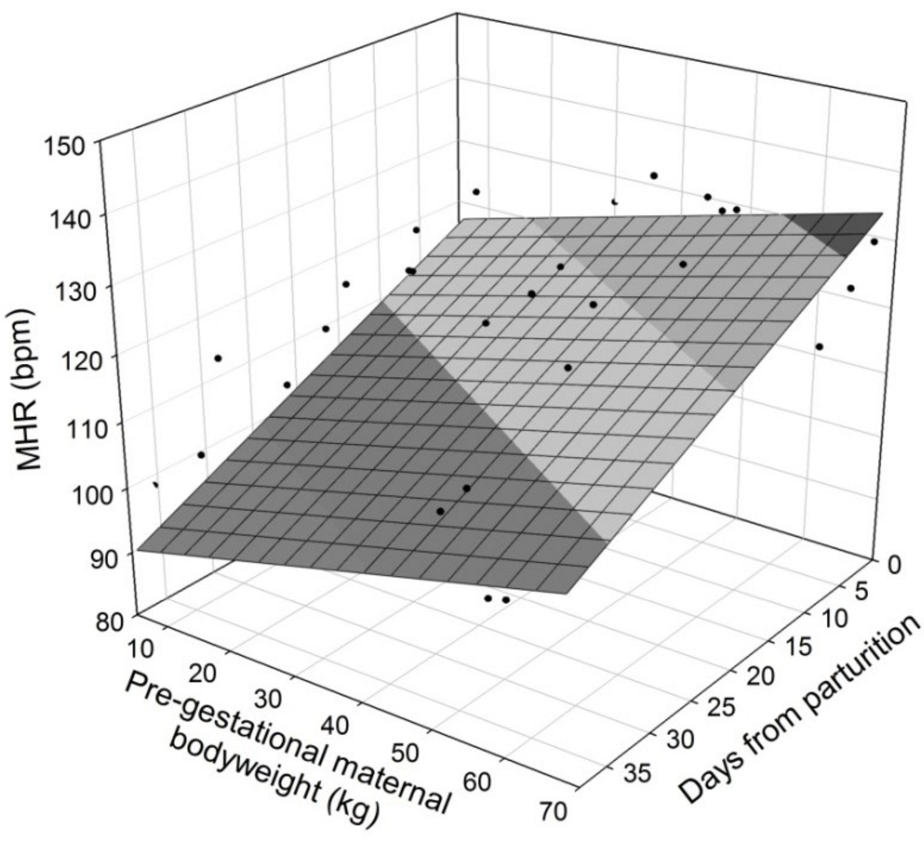
Table 11. *Coefficients for the quadratic regression of fetal heart rate (FHR) in bitches of different pre-gestational bodyweight at different gestational.*

	FHR coefficient	p	-95%	+95%
Intercept	211.6732	0.0001	202.9648	220.3817
Pre-gestational bodyweight	-0.6935	0.01	-1.2262	-0.1609
Pre-gestational bodyweight ^{2}	0.0100	0.016	0.0019	0.0182
Days from parturition	3.2479	0.0001	2.3645	4.1312
Days from parturition^2	-0.0810	0.0001	-0.1061	-0.0559

Significances and 95% confidence intervals are reported.

Data of MHR fitted significantly a linear regression equation ($p<0.01$ for all parameters, Fig. 8). Multiple r was 0.66, and the multiple $r^2=0.43$.

Figure 8. *Linear regression for maternal heart rate (MHR) in bitches as a function of pre-gestational bodyweight at different gestational age.*



The coefficients of the regression, the significance, and the confidence intervals are reported in Table 12. An increase of MHR was observed close to term and in bitches of high bodyweight.

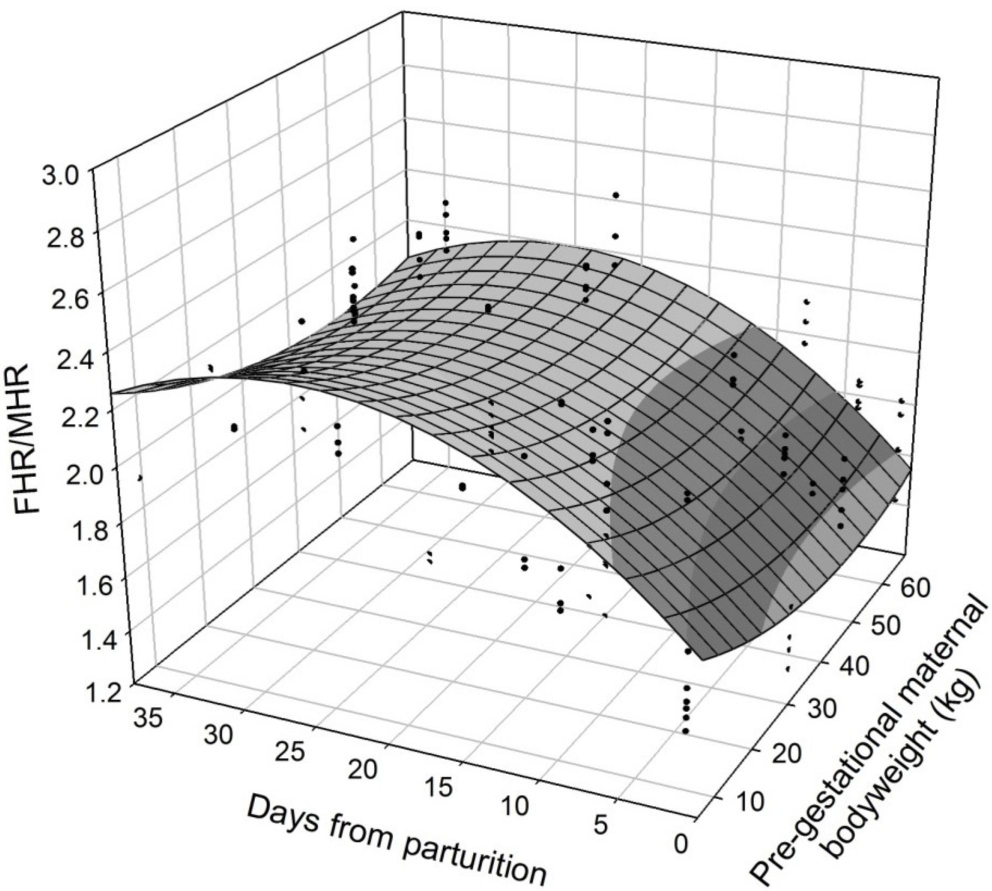
Table 12. *Coefficients for the linear regression of maternal heart rate (MHR) in bitches of different pre-gestational bodyweight at different gestational age.*

	MHR coefficient	p	-95%	+95%
Intercept	115.436	0.0001	100.896	130.178
Pre-gestational bodyweight	0.222	0.004	-0.123	0.595
Days from parturition	-0.600	0.001	-1.411	0.059

Significances and 95% confidence intervals are reported.

The ratio of FHR/MHR well fitted a multiple quadratic regression (saddle, Fig. 9), with all independent variables with significance at $p<0.05$. Multiple r was 0.71, with r^2 0.50.

Figure 9. *Polynomial quadratic regression for feto-maternal heart rate (FHR/MHR) ratio in bitches of different pre-gestational bodyweight at different gestational age.*



Therefore, FHR/MHR ratio was well expressed by a quadratic law, in terms of pre-gestational maternal bodyweight and gestational age. The coefficients of the regression, the significance, and the confidence intervals are reported in Table 13. As seen for FHR, the FHR/MHR ratio resulted higher in low and high bodyweight, and it reached the maximum values at about 20 days before parturition.

Table 13. *Coefficients for the quadratic regression of feto-maternal heart rate (FHR/MHR) ratio in bitches of different pre-gestational bodyweight at different gestational age.*

	FHR/MHR coefficient	p	-95%	+95%
Intercept	1.828405	0.0001	1.696833	1.959976
Pre-gestational bodyweight	-0.013705	0.001	-0.021753	-0.005658
Pre-gestational bodyweight ^{2}	0.000141	0.03	0.000017	0.000264
Days from parturition	0.050715	0.0001	0.037369	0.064061
Days from parturition ^{2}	-0.000986	0.0001	-0.001365	-0.000607

Significances and 95% confidence intervals are reported.

The equation derived from the quadratic regression was as follows:

$$z= 1.8284-0.0137x + 0.00014x^2+0.05071y-0.00099y^2$$

where z = FHR/MHR ratio, x = pre-gestational maternal bodyweight (kg), y= days before parturition.

GENERAL DISCUSSION

In clinical practice, for the proper management of pregnancy, it is crucial to make an accurate assessment of the development, viability and health of the fetus to allow an early detection of complications and to perform an adequate planning of caesarean section when the pregnancy is considered at risk.

Ultrasonographic evaluation of the fetal development

The lack of specific fetal growth curves in large and giant dogs prompted this investigation. Data (**Paper 1**) confirmed that ICC and BP are reliable indicators of the gestational age, as proved by the coefficients of determination (r^2) greater than 0.9.

The overall accuracy of the prediction (±1 day and ±2 days) that ranged between 62% and 89% is comparable with the accuracy previously obtained in small and medium dogs by size-related growth curves (Beccaglia & Luvoni, 2006). The application of specific formulas for giant dogs increased the BP accuracy compared to what reported in the literature by using non-specific curves (Socha & Janowski, 2014).

The accuracy at ±2 days of both parameters was significantly higher than that at ±1 day. This result was foreseeable as, with the extension of the time range, there is an increase of the probability that the actual and the predicted dates will fall within the same time range. Anyhow, 2 days between actual and predicted parturition term might be considered safe and acceptable in clinical practice.

In large bitches, as in small and medium size dogs (Beccaglia & Luvoni, 2006), both ICC and BP were equally reliable for the prediction of the delivery day, whereas in giant dogs the prediction should be preferably performed by ICC whose accuracy (±1 day and ±2 days) was significantly higher than that of BP.

This result differs from what has been observed in the aforementioned study in which no differences were found in the accuracy of these parameters in large and giant dogs when formulas for medium dogs were applied to a small number of observations (Socha & Janowski, 2014).

The inner chorionic cavity may be less affected than BP by individual variability of fetal growth during late gestation (Son et al., 2001; Kutzler et al., 2003; Beccaglia & Luvoni, 2006; Lopate, 2008; Socha & Janowski, 2014) and this could also explain the effect of litter size on the accuracy of BP measurements.

In small litters of giant dogs, the lower BP accuracy might have been related to the fetuses overgrowth and/or to the consequent gestation length prolongation (Gavrilovic et al., 2008). Litter size and duration of gestation are negatively correlated (Okkens et al., 1993; Okkens et al., 2001) and in giant dogs, where normal numbers of fetuses is generally higher than in large dogs (Sverdrup Borge et al., 2011), the effect of the presence of few fetuses could be more evident.

As previously observed in other sizes dogs (Beccaglia & Luvoni, 2006), the gender did not affect the accuracy of the prediction and this result is further confirmed by the observation that neonatal sex does not influence newborn bodyweight (Alonge et al., 2014; Groppetti et al., 2015b).

Evaluation of fetal health

Fetal heart rate (FHR) is a good indicator of fetal well-being, but its regulatory mechanisms and variability along pregnancy are still poorly understood. As previously mentioned, fetal distress is the main cause of FHR alteration, but other factors such as gestational age and pre-gestational maternal bodyweight should be taken into account.

Present results (**Paper 2**) demonstrate that in all bitches the FHR increased during pregnancy until 20 days before parturition and then a reduction was observed toward the term. These results are in agreement with those previously reported in an experimental colony of Beagles where an increase from 214+/-13.3 to 238.2+/-16.1 bpm at day 40 of pregnancy and a decrease to 218+/-6.7 bpm close to term were observed (Verstegen et al., 1993).

The fetal heart rate trend during pregnancy could be explained by the dynamics of the circulatory system maturation. Both in human and veterinary medicine, FHR trend is mainly related to the autonomic nervous system development and activity (Verdurmen et al., 2013). The neurotransmitters, noradrenaline and acetylcholine, influence the depolarization of the pacemaker cells of the heart, directly affecting the heart rate. The cardiac innervation ontogeny was studied using different techniques and in different species: chicken embryos, laboratory animals, sheep and dogs and finally humans (Papp, 1988; Long & Henry, 1992; Rosen & Danilo, 1992). All authors agree on the correlation between the FHR performance and the functional activity of nervous transmission during embryonic and fetal development. Sympathetic and parasympathetic control of circulatory functions mature at different rates during fetal development and the former becomes active earlier in fetal life, than the latter (Assali et al., 1977; Woods et al., 1977). For this reason, when the FHR is mainly under the sympathetic effect, its values are higher than at the end of pregnancy, when the development of the parasympathetic system occurs.

Even though the FHR trend is similar in dogs of different size, in low and high bodyweight bitches, the values were higher. In small dogs this finding might be related to their physiological higher sympathetic tone, whereas it was unexpected in heavy dogs, characterized by a prevalence of the parasympathetic tone (Hezzell et al.m 2013). It could be hypothesized that in these dogs the immaturity of parasympathetic system and the early development of the sympathetic system might be responsible of the higher FHR.

Concerning MHR, present results demonstrated an increase during pregnancy likely to provide adequate blood supply to the fetuses, as already reported in the literature (Lucio et al., 2009). Since the MHR could have been conditioned by the stress induced by the restraint and by the typical temperament of breeds, dogs of different breeds were included in the present study and the average values of three MHRs recorded during the same exam were considered.

Some authors emphasized the mutual influence between MHR and FHR during pathological events of the mother (shock or electrolyte imbalances) or during administration of drugs that cross the placenta (Eisenberg de Smoler et al., 1975). Therefore, it would seem reasonable to assume that they are also mutual influenced under physiological conditions.

The relationship between MHR and FHR was evaluated in pregnant women monitored for 24 hours and the results showed that the lowest and the highest MHRs corresponded to the FHRs. This was a confirmation of the mutual influence of the heart rates. The observation in women and sheep that FHR and MHR follow a circadian pattern has been considered a further evidence of this influence (Patrick et al., 1981, 1982; Bocking et al., 1985).

To evaluate the effective correlation between fetal and maternal heart rate in humans, some authors have included in their studies only pregnancies without complications, and data were divided into three groups based on the MHR: normal (71-92 bpm), tachycardic (107-155 bpm) and bradycardic (48-62 bpm). The comparison of the groups showed that the FHR values remain constant regardless of the different MHR. Conversely, it has been recently demonstrated that the maternal and fetal cardiovascular systems, even though strictly connected, are independent, and the fetus can respond to the maternal circulation oscillations (Van Leeuwen et al., 2009).

Similarly to FHR, the FHR/MHR ratio has reached the maximum values at about twenty days before birth and was higher in bitches of small and large bodyweight. The trends of FHR and FHR/MHR ratio were similar, but the ratio better described the effect of the independent variables, such as maternal bodyweight and gestational age, on the data ($r^2 = 0.50$ vs. $r^2 = 0.25$). The non-linear trend of the ratio, (i.e. increase until 20 days before parturition followed by a remarkable decrease) suggests that in the late pregnancy the fetus may be able to manage, at least partially, maternal cardiovascular fluctuations, as observed in women and ewes. In this period MHR increased linearly, whereas FHR and the ratio decreased toward the term to ensure the best subsistence of the conceptus (Eisenberg de Smoler et al., 1975; Bocking et al., 1985; Van Leuwen et al., 2003; Van Leuwen et al., 2009).

In this study, only data derived from uncomplicated pregnancies were analyzed, and such strict recruitment had the purpose to exclude any possible alteration related to pathological conditions of the fetuses that could influence the FHRs and therefore their correlation with the MHR.

Among factors that affect FHR and FHR/MHR ratio, fetal movements should be considered. They have been deeply investigated in women and large animals (Dawes et al., 1992; Di Renzo et al., 1994a; Baska-Vinze et al., 2014), in which changes in FHR according to normal rates of movements have been described. Few information are available in dogs in which temporary accelerations of FHR may be associated with fetal movements (Davidson, 2003), but normal rates of fetal activity during different pregnancy periods, have not yet been defined. Therefore, a potential effect of fetal movements on FHR deserves further investigations in this species. It remains also to evaluate the potential effect of the litter size on FHR and on FHR/MHR ratio.

CONCLUSIONS

In clinical practice, for the proper management of pregnancy, an accurate assessment of fetal development and health is crucial to achieve an early diagnosis of complications.

A highly accurate assessment of the fetal development is obtained by the ultrasonographic fetal biometry. The enormous variety in size among different canine breeds prompts the use of specific size-related formulae, which ensure an accurate identification of the gestational age. The ultrasonographic evaluation of fetal development in large and giant bitches completes and concludes the study of size-related formulae for clinical application in all different size dogs (**Paper 1**).

Among the few parameters available in the literature, fetal heart rate is generally used to objectively evaluate the fetal health during canine pregnancy. Results of the present study suggest that the maternal pre-gestational bodyweight and the gestational age influence both FHR and FHR/MHR ratio. The highest significance of FHR/MHR ratio, compared to FHR, encourages the application of this ratio to evaluate fetal health. For this reason the obtained equation for FHR/MHR ratio (z + = 1,8284-0,0137x 0,00014x2 + 0,05071y-0,00099y2 where z = relationship FHR/MHR, x = maternal weight before pregnancy (Kg), y = days until parturition), that describes the trend in healthy fetuses, could be helpful in clinical practice to derive expected values in uncomplicated pregnancies (**Paper 2**).

FUTURE PERSPECTIVES

The definition of reliable criteria for fetal monitoring in mammals is needed to set up therapeutic interventions along pregnancy and to possibly prevent irreversible damages. The analysis of the recent literature shows the current attempt to adapt guidelines and knowledges of human medicine and make them applicable to veterinary obstetrics and gynecology.

Since the '80s in human medicine, for the evaluation of fetal well-being the fetal biophysical profile (BPP) was introduced. The BPP consists in the ultrasound evaluation of five fetal variables: breathing movements, body movements, muscle tone, amniotic fluid index, heart rate reactivity with temporary acceleration in response to body movements. To each criterion is assigned a score from 0 to 2 and the normal overall range is set between 8 to 10 points; under 8 points the BPP might indicate fetal stress, morbidity and perinatal mortality (Manning et al., 1980, 1984, 1985, 1986, 1987; Manning, 1990).

In veterinary medicine the application of BPP has been tested in horses and cattle.

In the horse, with transabdominal ultrasonography, a BPP has been codified by six different ultrasound parameters: FHR, fetal aortic diameter, fetal fluid maximal depth, fetal activity, uteroplacental contact and thickness (Reef et al., 1995, 1996; Reef, 1998). This profile may give indications of impending birth and possible complications. Unfortunately, predictive values of the BPP are not as reliable as in humans and its limited sensitivity and specificity in horses are primarily due to the choice of the selected parameters. For instance, in clinical practice the detection of the aortic diameter and fetal breathing movements is hard and time consuming (Palmer, 2000; Baska-Vincze et al., 2014).

The application of the BPP in cattle, as described by Reef and colleagues (1996) in the horse, did not give the expected results. The fetal activity is linked to the health of the conceptus, but the bovine fetus lives long periods of rest. During the ultrasound examination, which generally lasts around 10 minutes, body movements of healthy fetuses can be totally absent (Buczinski et al., 2009).

A different parameter that can be evaluated in cattle is the fetal weight through the measurement of aortic diameter. In Holstein cows a growth retardation (Intra Uterine Growth Restriction; IUGR) or the presence of large fetuses frequently occur; these two conditions can cause complications during delivery and inauspicious outcomes. However, for some authors, the prediction of the birth weight of the calf from the diameter of the aorta raises some critical issues (Buczinski et al., 2007; Baska-Vincze et al., 2014).

In dogs, fetal BPP has not yet been described. As reported above, one of the main limits of this technique might be represented by the choice of species-specific parameters to obtain a reliable BPP for the early detection of pregnancy alterations. Other indicators of fetal development and health, other than ICC or BP, FHR and FHR/MHR ratio, should be identified and analyzed in bitches.

However, it should not be disregarded that the dog is a polytocic species. This limits the possibility of an ultrasonographic recognition of individual fetuses. Only in early pregnancy the embryos might be individually identified, and specific ultrasonographic indicators could be considered.

It is important to underline that in the same pregnancy suffering/dead and healthy fetuses could be concurrently present. The decision to perform medical or surgical therapy has to be supported by a careful evaluation of risks and benefits that might be difficult to estimate.

REFERENCES

Abbott J. A. (2010) *The effect of pregnancy on echocardiographic variables in healthy bitches*. Journal of Veterinary Cardiology, 12:123-128

Alonge S., Meloni T., Melandri M., *et al.* (2014) *Factors influencing birth weight and neonatal weight gain in Chow Chow puppies*. Proceedings of the 17th EVSSAR (European Veterinary Society for Small Animal Reproduction) Congress, Wroclaw, Poland. p 164

Alonge S., Beccaglia M., Melandri M., *et al* (2015) *Prediction of parturition term in large and giant size bitches by ultrasonographic measurements*. Proceedings of the 18th EVSSAR (European Veterinary Society for Small Animal Reproduction) Congress, Hannover, Germany. p 145

Arbeiter K. (1993) *Anovulatory ovarian cycles in dogs.* Journal of Reproduction and Fertility, 47S:453-456

Assali N.S., Brinkman C. R., Woods J. R., *et al*. (1977) *Development of neurohumoral control of fetal, neonatal, and adult cardiovascular functions*. American Journal of Obstetrics and Gynecology, 129:748-759

Bader R.A., Bader M.E., Rose D.J., *et al.* (1955) *Hemodynamics at rest and during exercise in normal pregnancy as studies by cardiac catheterization*. Journal of Clinical Investigation, 34:1524-1536

Baska-Vincze B., Baska F., Szenci O. (2014) *Transabdominal ultrasonographic evaluation of fetal well-being in the late-term mare and cow*. Acta Veterinaria Hungarica, 62:439-451

Beccaglia M., Luvoni G.C. (2004) *Ultrasonographic study during pregnancy of the growth of an encephalic portion in the canine foetus*. Veterinary Research Communication, 28:161-164

Beccaglia M., Luvoni G.C. (2006) *Comparison of the accuracy of two* *ultrasonographic measurements in predicting the parturition date in the bitch.* Journal of Small Animal Practice, 47:670–673

Beccaglia M., Faustini M., Luvoni G.C. (2008) *Ultrasonographic study of deep portion of diencephalo-telencephalic vescicle for the determination of gestational age of the canine foetus*. Reproduction in Domestic Animal, 43:367-370

Beccaglia M., Luvoni G.C. (2012) *Prediction of Parturition in Dogs and Cats: Accuracy at Different Gestational Ages.* Reproduction in Domestic Animals, 47:194-196

Blanco P.G., Arias D.O., Gobello C. (2008) *Doppler ultrasound in canine pregnancy*. Journal of Ultrasound in Medicine, 27:1745-1750

Blanco P.G., Arias D., Rube A., *et al.* (2009) *An experimental model to study resistance index and systolic/diastolic ratio uterine arteries in adverse canine pregnancy outcome*. Reproduction in Domestic Animals, 44:164-166

Blanco P.G., Tórtora M., Rodriguez R., *et al.* (2011) *Ultrasonographic assessment of maternal cardiac function and peripheral circulation during normal gestation in dogs*. The Veterinary Journal, 190:154-159

Bocking A.D., Harding R., Wickham P.J. (1985) *Relationship between accelerations and deceleration in heart rate and skeletal muscle activity in sheep.* Journal of Developmental Physiology, 7:47-54

Boddy K., Dawes G.S., Fisher R., *et al.* (1974) *Foetal respiratory movements, electrocortical and cardiovascular responces to hypoxaemia and hypercapnia in sheep*. The Journal of Physiology, 243:599-618

Bodey A.R., Michell A.R. (1996) *Epidemiological study of blood pressure in domestic dogs*. The Journal of Small Animal Practice, 37:116-125

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., *et al.* (2004) *Transrectal Doppler sonography of uterine and umbilical blood flow during early pregnancy in mares*. Theriogenology, 60:597-605

Boroffka S.A. (2005) *Ultrasonographic evalutation of pre- and postnatal development of the eyes in beagles*. Veterinary Radiology and Ultrasound, 46:72-79

Brooks V.L., Keil L.C. (1994a) *Changes in the baroreflex during pregnancy in conscious dogs: heart rate and hormonal responses*. Endocrinology, 135:1894-1901

Brooks V.L., Keil L.C. (1994b) *Hemorrage decreases arterial pressure sooner in pregnant compared with nonpregnant dogs: role of baroreflex*. American Journal of Physiology, 266:1610-1619

Buczinski S., Fecteau G., Lefebvre R.C., *et al.* (2007) *Fetal well-being assessment in bovine near-term gestations: current knowledge and future perspectives arising from comparative medicine*. The Canadian Veterinary Journal, 48:178-183

Buczinski S., Fecteau G., Comeau G., *et al*. (2009) *Ultrasonographic fetal well-being assessment, neonatal and post-partum finding a preliminary study on 10 fetuses and calves*. The Canadian Veterinary Journal, 50: 261-269

Concannon P.W., McCann J.P., Temple M. (1989) *Biology and endocrinology of ovulation, pregnancy and parturition in the dog*. Journal of Reproduction and Fertility, 39S:3-25

Concannon P.W. (2000) *Canine pregnancy: predicting parturition and timing events of gestation*. International Veterinary Information Service; www.ivis.org.

Concannon P.W. (2003) *Ultrasound imaging of reproductive tract of the bitch.* International Veterinary Information Service; www.ivis.org.

Davidson A.P. (1998) *Uterine monitoring during pregnancy*. Proceedings of the Annual Meeting of the Society for Theriogenology, Baltimore, USA. p 123-125

Davidson A.P. (2001) *Uterine and fetal monitoring in the bitch*. The Veterinary Clinics of North America. Small Animal Practice, 31:305-313

Davidson A.P. (2003) *Obstetrical monitoring in dogs*. Veterinary Medicine, 6:508-516

Davidson A.P., Eilts B. (2006) *Advanced Small Animal Reproductive Techniques*. Journal of the American Animal Hospital Association, 42:10-17

Davidson A.P. (2008) *Dystocia management*. Current Veterinary Therapy, 13:992-998

Dawes G.S., Fox H.E., Leduc B.M. (1972) *Respiratory movements and rapid eye movements sleep in the foetal lamb*. The Journal of Physiology, 220:119-143

Dawes G.S., Moulden M., Redman C.W. (1992) *Short-term fetal heart rate variation, decelerations, and umbilical flow velocity waveforms before labor*. Obstetrics and Gynecology, 80:673-678

DeVries J.I.P., Visser G.H.A., Prechtl H.F.R. (1982) *The emergence of fetal behaviour*. Early human Development, 7:301-322

Di Renzo G.C., Copray F.J.A., O'Herlihy C., *et al.* (1994a) *Maternal-fetal surveillance within the European community.* In: Van Gejin HP, Copray FA (Eds). A critical appraisal of fetal surveillance. Amsterdam: Elsevier Science, p 11-15.

Di Renzo G.C., Luzi G., Caserta G., *et al*. (1994b) *The role of telemetry in perinatal monitoring*. Journal of perinatal medicine, 22:517-522

Di Salvo P., Bocci F., Zelli R., *et al.* (2006) *Doppler evalutation of maternal and fetal vessels during normal gestation in the bitch*. Research in Veterinary Science, 81:382-388

Dubiel M., Breborowicz G.H., Gudmundsson S. (2003) *Evalutation of fetal circulation redistribution in pregnancies with absent or reversed diastolic flow in umebelical artery*. Early Human Development, 71:149-156

Eisenberg de Smoler P., Smith H.C., Karchmer S. (1975) *Correlation of fetal heart rate, maternal heart rate, and age of pregnancy*. American Journal of Obstetrics and Gynaecology, 121:62-65

England G.C.W., Allen W.E., Porter D.J. (1990) *Studies on canine pregnancy using B-mode ultrasound: develompent of the conceptus and determination of gestational age.* Journal of Small Animal Practice, 31:324–329

England G.C.W., Russo M. (2006) *Ultrasonographic characteristic of early* *pregnancy failure in bitches.* Theriogenology, 66:1694–1698

Fischer W.M. (1979) *Fondamenti e valore clinico della cardiotocografia*. Testo Atlante di cardiotocografia. Piccin Editore, Milano, Italia.

Fleischer A.C., Emerson D.S. (1994) *Ecografia Doppler a colori in Ostetricia e Ginecologia*. Salerno, Momento Medico s.r.l., Salerno, Italia. p 1-15

Gavrilovic B.B., Andersson K., Linde Forsberg C. (2008) *Reproductive patterns in the domestic dog--a retrospective study of the Drever breed.* Theriogenology 70:783-794

Gil E.M., Garcia D.A., Giannico A.T., et al. (2014) *Canine fetal heart rate: do accelerations or decelerations predict the parturition day in bitches?* Theriogenology. 82, 933-941

Götze R. (1955) *La gravidanza normale nella sua evoluzione terminale*. In: Manuale di Ostetricia, Richter G., Götze R.(Eds). Edizione italiana di Borrelli G., Casa editrice Palmiero, Chieti

Groppetti D., Vegetti F., Bronzo V., *et al.* (2015a) Breed-specific fetal biometry and factors affecting the prediction of whelping date in the German shepherd dog. *Animal Reproduction Science* 152:117-122

Groppetti D., Ravasio G., Bronzo V., *et al.* (2015b) The role of birth weight on litter size and mortality within 24h of life in purebred dogs: What aspects are involved? *Animal Reproduction Science* 163:112-119

Hamlin R.L. Olsen I., Smith C. R., *et al.* (1967) *Clinical relevancy of heart rate in the dog*. Journal of the American Veterinary Medical Association, 151:30-63

Hezzell M.J., Humm K., Dennis S.G., *et al.* (2013) *Relationships between heart rate and age, bodyweight and breed in 10849 dogs*. Journal of Small Animal Practice, 54:318-323

Johnston S.D. (1983) *Prenatal indicators of puppy viability at term*. Compendium and continuing education for practice veterinary 5:1113-1125

Johnston S.D., Root Kustritz M.V., Olson P.N. (2001) *Canine pregnancy*. In: Canine and Feline Theriogenology; Johnston S.D., Root Kustritz M.V., Olson P.N(Eds), W.B. Saunders Company, Toronto, p 66-104

Khodiguian N., Jaque-Fortunato S., Wiswell R.A., *et al.* (1996) *A comparison of cross-sectional and longitudinal methods of assessing the influence of pregnancy on cardiac function during exercise*. Seminars in Perinatology, 20:232-241

Kim B., Son C. (2007) *Time of initial detection of fetal and extra-fetal structures by ultrasonographic examination in Miniature Schnauzer bitches.* Journal of Veterinary Medical Science, 8:289–93.

Kim Y., Travis A.J., Meyers-Wallen V.N., et al. (2007) *Parturition prediction and timing of canine pregnancy*. Theriogenology, 68:1177-1182

Konde L. (1988) *Diagnostic ultrasound in canine pregnancy and uterine disease*. Theriogenology, 17:247-249

Kutzler M.A., Yeager, A.E., Mohammed, H.O., *et al.* (2003) *Accuracy of canine parturition date prediction using fetal measurement obtained by ultrasonography.* Theriogenology 60:1309–1317

Lamm C., Makloski C.L. (2012) *Current advance in gestation and parturition in cats and dogs*. The Veterinary Clinics of North America. Small Animal Practice, 42:445-456

Lévy X., Fontbonne A. (2007) *Determining the optimal time of mating in bitches: particularities.* Revista Brasileira de Reproduo Animal, 31:128-134

Long W.A., Henry G.W. (1992) *Autonomic and central neuroregulation of fetal cardiovascula function*. In: Fetal and neonatal physiology, W.B. Saunders Company, Toronto, Canada. p 629-645

Lopate C. (2008) *Estimation of gestational age and assessment of canine fetal maturation using radiology and ultrasonography: a review.* Theriogenology 70:397–402

Lucio C.F., Silva L.C.G., Rodrigues J.A., *et al.* (2009) *Peripartum haemodynamic status of bitches with normal birth or dystocia.* Reproduction in Domestic Animals, 44:133-136

Luvoni G.C., Grioni A. (2000) *Determination of gestational age in medium and small size bitches using ultrasonographic fetal measurements*. Journal of Small Animal Practice, 41:292-294

Luvoni G.C., Beccaglia M. (2006) *The prediction of parturition date in canine pregnancy.* Reproduction in Domestic Animals, 41:27–32

Lenard ZM, Hopper BJ, Lester NV, *et al*. (2007) *Accuracy of prediction of canine litter size and gestational age with ultrasound*. Australian Veterinary Journal, 85: 222–225

Manning F.A., Platt L.D., Sipos L. (1980) *Antenatal fetal evalutation. Development of a fetal biophysical profile*. American Journal of Obstetrics and Gynecology, 136:787-795

Manning F.A., Lange I.R., Morrison I., *et al.* (1984) *Fetal biophysical profile score and the nonstress test: a comparative trial*. Obstetrics and Gynaecology, 64:326-331

Manning F.A., Morrison I., Lange I.R., *et al*. (1985) *Fetal assessment based on fetal biophysical profile scoring: experience in 12,620 referred high-risk pregnancies*. American Journal of Obstetrics and Gynecology, 151:343-350

Manning F.A., Harman C.R., Lange I.R., *et al.* (1986) *Fetal assessment by biophysical profile scoring: 1985 update*. European Journal of Obstetrics, Gynecology and Reproductive Biology, 21:331-339

Manning F.A., Morrison I., Lange I.R., *et al.* (1987) *Fetal biophysical profile scoring: selective use of the nonstress test*. American Journal of Obstetrics and Gynecology, 156:709-712

Manning F.A. (1990) *The use of sonography in the evalutation of the high-risk pregnancy*. The Radiology Clinics of North America, 28:205-216

Maulik D. (1999) *Eco Doppler in ostetricia e ginecologia*. Mulik D. (Eds) CIC Edizioni Internazionali, Roma, Italia

Michel E., Spörri M., Ohlerth S., *et al.* (2011) *Prediction of parturition date in the bitch and queen.* Reproduction in Domestic Animals, 46:926–932

Miles K. (1995) *Imaging pregnant dogs and cats*. Compendium Continuing Education Practice Veterinary, 17:1217-1226

Mone S. M., Sanders S. P., Colan S. D. (1996) *Control mechanisms for physiological hypertrophy of pregnancy*. Circulation, 94:667-672

Moriyoshi M., Waki Y., Nakao T., *et al.* (1996) *Observation of the growth of beagle embryo and fetus by ultrasonography*. The Journal of Veterinary Medical Science, 58:443-445

Nautrup C.P. (1998) *Doppler ultrasonography of canine maternal and fetal arteries during normal gestation*. Journal of Reproduction and Fertility, 112:301-314

Nicolaides K.H., Rizzo G., Hecher K. (2000) *Placental and Fetal Doppler*. The Parthenon Publishing Group, Casterton Hall Carnforth Lancs (UK), p. 35-66

Nyland T.G, Matton J.S. (2002) *Ovaries and uterus*. In: Small animal diagnostic ultrasound. WB Saunders Co. p. 231–49

Okkens A., Hekerman T., de Vogel J., *et al.* (1993) *Influence of litter size and breed on variation in length of gestation in the dog.* The Veterinary Quarterly 15,160-161

Okkens, A., Tenissen, J., Van Osch, W., *et al.* (2001) *Influence of litter size and breed on the duration of gestation in dogs.* Journal of Reproduction and Fertility 57S:193-197

Palmer J. (2000) *Fetal monitoring*. Proceedings of the Equine Symposium and Annual Conference, San Antonio, USA. p. 39-43

Papp J.G. (1988) *Autonomic rasponses and neurohumoral control in the human early antenatal heart*. Basic Research in Cardiology, 83:2-9

Patrick J., Campbell K., Carmichael L., *et al.* (1981) *Daily relationship between fetal and maternal herat rates at 38 to 40 weeks of pregnancy*. Canandian Medical Association Journal, 124:1177-1178

Patrick J., Campbell K., Carmichael L. (1982) *Influence of maternal heart rate and gross fetal body movements on the daily pattern of fetal heart rate near term*. American Journal of Obstetrics and Gynecology, 144:533-538

Patrick J., Challis J.R.G., Cross J. (1987) *The relationship between fetal breathing movements and prostaglandin F2 during ACTH-induced labour in sheep*. Journal of Developmental Physiology, 9:287-294

Patrick J. (1989) *The physiological basis for fetal assessment*. Seminars in Perinatology, 5:403-408

Poffenbarger E., Feeney D.A. (1986) *Use of gray-scale ultrasonography in the diagnosis of reproductive disease in the bitch*. Journal of the American Veterinary Medical Association, 189:90-95

Reed K.L., Chaffin D.L., Anderson C.F. (1996) *Umbelical Venous Doppler Velocity pulsations and inferior Vena Cava Pressure Elevations in Fetal lambs*. Obstetrics and Gynaecology, 8:617-620

Reef V.B., Vaala W.E., Worth L.T. *et al.* (1995) *Ultrasonographic evaluation of the fetus and intrauterine environment in healthy mares during late gestation*. Veterinary Radiology and Ultrasound, 36:533–541

Reef V.B., Vaala W.E., Worth L.T., et al. (1996) *Ultrasonographic assessment of fetal well-being during late gestation: development of an equine biophysical profile*. Equine Veterinary Journal, 28:200-208

Reef V.B. (1998) *Equine diagnostic ultrasound*. W.B. Saunders Company, Philadelphia. p. 560

Rendano V.T. (1983) *Radiographic evalutation of fetal development in the bitch and fetal death in the bitch and queen*. Current Veterinary Therapy, 8:947-952

Robson S.C., Hunter S., Boys R., *et al.* (1989) *Serial study of factors influencing changes in cardiac output during human pregnancy*. American Journal of Obstetrics and Gynecology, 256:1060-1065

Root-Kustritz M.V. (2005) *Pregnancy diagnosis and abnormalities of pregnancy in the dog*. Theriogenology, 64:755-765

Rosen M.R., Danilo P.J. (1992) *Developmental electrophysiology of the heart*. Fetal and neonatal physiology, W.B. Saunders Company, Philadelphia, 1992, vol. 1, p. 656-665

Ruckebusch Y. (1972) *Development of sleep and wakefulness in the foetal lamb*. Clinical Neurophysiology, 32:119-128

Socha P., Rudowska M., Janowski T. (2012) *Effectiveness of determining the parturition date in bitches using the ultrasonographic fetometry as compared to hormonal and cytological methods.* Polish Journal of Veterinary Science, 3:447–53

Socha P., Janowski T. (2011*) Predicting the parturition date in Yorkshire Terrier and Golden Retriever bitches using ultrasonographic fetometry*. Bulletin of the Veterinary Institute in Pulawy, 55:71–5

.Socha P., Janowski T. (2014) *Predicting the parturition date in bitches of different body weight by ultrasonographic measurements of inner chorionic cavity diameter and biparietal diameter*. Reproduction in Domestic Animals, 49:292–296

Socha P., Janowski T., Bancerz-Kisiel A. (2015) *Ultrasonographic fetometry formulas of inner chorionic cavity diameter and biparietal diameter for medium-sized dogs can be used in giant breeds.* Theriogenology, 84:779–783

Son C., Jeong K., Kim J., *et al.* (2001) *Establishment of prediction table of parturition day with ultrasonography in small pet dogs.* The Journal of Veterinary Medical Science, 7:715–721

Spatling L., Fallenstein F., Huck A. (1992) *The variabililty of cardiopulmonary adaptation to pregnancy at rest and during exercise*. British Journal of Obstetrics and Gynaecology, 99:1-40

Sridevi P. (2013) *Ultrasonographic diagnosis and monitoring of pregnancy in the bitch, a review*. Journal of Veterinary Science, 44:1-7

Sverdrup Borge K., Tønnessen R., Nødtvedt A., *et al.* (2011) *Litter size at birth in purebred dogs—A retrospective study of 224 breeds.* Theriogenology 75:911–919

Toal R.L., Walker M.A., Henry G.A. (1986) *A comparison of real-time ultrasound, palpation and radiography in pregnancy detection and litter size determinantion in the bitch*. Veterinary Radiology, 27:102-108

Tønnessen R., Sverdrup Borge K., Nødtvedt A., *et al.* (2012) Canine perinatal mortality: A cohort study of 224 breeds. *Theriogenology* 77:1788–1801

Valensise H., Novelli G.P., Vasapollo B., *et al.* (2000) *Maternal cardiac systolic and diastolic function: relationship with uteroplacental resistances*. Ultrasound in Obstetrics and Gynecology, 15:487-497

Van Leeuwen P, Geue D, Lange S, *et al.* (2003) *Is there evidence of fetal-maternal heart rate synchronization?* BMC Physiol 3:2

Van Leeuwen P., Geue D., Thiel M., *et al.* (2009) *Influence of paced maternal breathing on fetal-maternal heart rate coordination*. Proceedings of the National Academy of Sciences, 106:13661-13666

Verdurmen K.M.,Renckens J., Van Laar J.O., *et al.* (2013) *The influence of corticosteroids on fetal heart rate variability: a systematic review of the literature*. Obstetrics and Gynecology Survey, 68:811-824

Verstegen J.P., Silvia L.D.M., Onclin K., *et al.* (1993) *Echocardiographic study of heart rate in dog and cat fetuses in utero*. Journal of Reproduction and Fertility. 47S:174-180

Williams J.C., Rincon-Skinner T., Sun D., *et al.* (2007) *Role of nitric oxide in the coupling of myocardial oxygen consumption and coronary vascular dynamics during pregnancy in the dog*. American Journal of Physiology, 293:2479-2486

Woods J.R., Dandavino A., Murayama K., *et al.* (1977) *Autonomic control of cardiovascular functions during neonatal development and in adult sheep*. Circulation Research, 40:401-407

Yeager A.E., Concannon P.W. (1990) *Association between the preovulatory luteinizing hormone surge and the early ultrasonographic detection of pregnancy and fetal heartbeats in Beagle dogs.* Theriogenology 34:655-665

Yeager A.E., Mohammed H.O., Meyers-Wallen V., *et al.* (1992) *Ultrasonographic appearance of the uterus, placenta, fetus and fetal membranes throughout* *accurately timed pregnancy in Beagles.* American Journal of Veterinary Research 3:342–351

Zone M.A., Wanke M.M. (2001) *Diagnosis of canine fetal health by ultrasonography.* Journal of Reproduction and Fertility 57S:215-219

SUMMARY

Nowadays ultrasonography represents the gold standard method for the evaluation of canine fetal development and health.

In dogs, the gestational age is estimated by the ultrasonographic measurements of extra-fetal and fetal structures. The fetal development has been deeply investigated in small and medium dogs, whereas only few information are available for large and giant size bitches, even though they are very popular canine breeds.

The measurements of the inner chorionic cavity (ICC) in early pregnancy, and the biparietal diameter (BP) in late pregnancy are commonly used in clinical practice. Both ICC and BP are highly reliable when size-related specific formulae are applied, thus, specific equations for large and giant dogs would allow the most accurate prediction of parturition term.

Only few parameters are available to objectively assess the fetal health during canine pregnancy. Among them, the fetal heart rate (FHR) is generally used, but the relationship between FHR and maternal heart rate (MHR) has been poorly investigated. The availability of reference values of the ratio FHR/MHR, could better contribute to the evaluation of the fetal health at different gestational ages, than the single FHR values.

The aims of this study were 1) to derive the growth curves of extra-fetal and fetal structures (ICC and BP) in large and giant size bitches and to evaluate their accuracy (**Paper 1**); 2) to evaluate the trend of FHR and of the ratio FHR/MHR in bitches of different pre-gestational bodyweight (**Paper 2**).

Present results showed a significant relationship between days before parturition and ICC or BP in large and giant size bitches. The overall accuracy ±2 days of both parameters was significantly higher than the accuracy ±1 day. Only in giant bitches, the BP accuracy of the prediction was significantly lower in small than normal litter size. As previously observed in other sizes dogs, the gender did not affect the accuracy of the prediction.

The second study demonstrated that both FHR and FHR/MHR significantly fitted a multiple quadratic regression for all independent variables. They both resulted higher in low and high bodyweight, and reached the maximum values at about 20 days before parturition. Maternal pre-gestational bodyweight and the gestational age influenced both FHR and FHR/MHR.

The highest significance of FHR/MHR, compared to FHR, encourages the application of this ratio, to evaluate fetal health. The derived equation for FHR/MHR ratio, that describes the trend in healthy fetuses, could be helpful in clinical practice to derive expected values in uncomplicated pregnancies.

PAPER 1

PREDICTION OF PARTURITION TERM IN LARGE AND GIANT SIZE BITCHES BY ULTRASONOGRAPHIC MEASUREMENTS

(submitted to Journal of Small Animal Practice)

PREDICTION OF PARTURITION TERM
IN LARGE AND GIANT SIZE BITCHES BY ULTRASONOGRAPHIC MEASUREMENTS

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Author contributions

SA and GCL contributed to design the study, collect and analyze the data, and draft the paper. MB and MM contributed to collect the data. 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.

STRUCTURED SUMMARY

Objectives. To derive the growth curves of the inner chorionic cavity (ICC) and the fetal biparietal diameter (BP) in large and giant size bitches by ultrasonographic measurements. To evaluate their accuracy in the prediction of parturition and the effect of litter size and fetal sex ratio.

Methods. Ultrasonographic examinations were performed in 8 large (26-40kg) and 9 giant (>40kg) pregnant bitches. The relationship between ICC or BP growth and days to parturition was analyzed by linear regression and the equations derived from growth curves were applied to predict the day of delivery. The accuracy of the prediction (±1 day and ±2 days) and the effect of litter size and sex ratio were assessed in large and giant bitches with unknown breeding dates.

Results. The results showed a significant relationship between days before parturition and ICC or BP. The overall accuracy at ±2 days was higher, than at ±1 day. In giant bitches, the accuracy of the prediction by BP was significantly lower in small, than normal litter size. No effect of fetal sex ratio was observed.

Clinical significance. Large and giant dogs are well represented in canine population and specific equations to predict the day of parturition might be useful in clinical practice.

Running head: ultrasonographic fetal biometry in large and giant bitches

Keywords: dog, ultrasonography, fetal biometry.

INTRODUCTION

Ultrasonographic fetal biometry is commonly applied for the assessment of fetal growth along pregnancy. In medium and small size dogs, the fetal development has been deeply investigated, and the measurement of extra-fetal and fetal structures has allowed the prediction of parturition day through equations generated from the growth curves of selected parameters (see reviews: Luvoni and Beccaglia 2006; Kim *et al.* 2007; Lopate 2008).

In the one hundred most popular canine breeds (Sverdrup Borge *et al.* 2011; Tønnessen *et al.* 2012), large and giant size bitches are very well represented. For these dogs, specific formulas for the evaluation of fetal growth are not yet available. Kutzler and colleagues (2003) suggested the use of a correction factor to adjust the difference between actual and predicted parturition date obtained with previously published equations for dogs of smaller size (England *et al.* 1990; Yeager *et al.* 1992).

Although the formulas for medium dogs (Luvoni and Grioni, 2000) have been also used in large and giant breeds (Socha and Janowski 2014; Socha *et al.* 2015), specific equations for these dogs would allow the most accurate prediction of parturition term (Michel *et al.* 2011; Socha and Janowski 2014; Socha *et al.* 2015).

Among several ultrasonographic parameters, the inner diameter of chorionic cavity (ICC) and the biparietal diameter (BP) provided a highly accurate prediction in small and medium size dogs (Luvoni and Grioni 2000; Kutzler *et al.* 2003; Beccaglia and Luvoni 2006; Socha and Janowski 2014). Therefore, the aim of this study was to derive the growth curves of ICC and BP in large and giant size bitches and to evaluate their accuracy. The effects of litter size and fetal sex ratio on the accuracy of the prediction were also investigated.

MATERIALS AND METHODS

Growth curves of ICC and BP in large and giant size bitches.

Eight large size (26-40 kg) bitches (Bergamasco Shepherd, Boxer, Doberman, German Shepherd, and Old English Shepherd) and 9 giant size (>40 kg) bitches (Great Dane, Bernese Mountain Dog, and Newfoundland), aged between 2 and 8 years presented to the Department for breeding management and pregnancy evaluation, were included in this study. Informed owner consent was obtained.

All bitches were healthy at the physical examination. For breeding management, the day of the ovulation was considered to be when plasma progesterone concentration ranged between 4-10 ng/ml (Arbeiter 1993; Lévy and Fontbonne 2007), as evaluated using an Enzyme Linked Fluorescent Assay (MiniVidas, BioMerieux, Marcy l'Etoile, France). Serial ultrasonographic exams were performed weekly from day 20 after mating until parturition. Bitches were positioned in lateral recumbency, transmission gel was applied, and two-dimensional, gray-scale, real-time ultrasound images were produced using a 7.5 MHz microconvex probe (SonoAce 8800, Medison Co. Ltd., Seoul, Korea). During early pregnancy, inner diameter of chorionic cavity, and in late pregnancy the biparietal diameter were measured. At least three measurements of ICC or BP, according to the gestation period, were recorded and the mean values were calculated. The time of actual parturition, the litter size, and the sex of the puppies were reported by the owners.

Statistical analysis: the relationship between ICC or BP growth and days before parturition was analyzed by a linear regression model. The growth equations for both parameters were derived as y=a+bx (y=days before parturition, x=measurement in mm of ICC or BP, a=intercept coefficient and b=first order coefficient) and the regression coefficients were analyzed by the Student's T test ($p<0.05$). Data analysis was performed with the software Stat Plus 2009.

Accuracy of the ICC and BP for the prediction of parturition day in large and giant size bitches.

To assess the accuracy of the prediction, measurements of ICC and BP were performed in 65 and 102 ultrasound examinations of large size bitches and in 39 and 52 of giant size bitches with unknown breeding dates. As previously reported, the prediction was considered accurate when the difference between actual and predicted parturition date was within ±1 day and ±2 days (Beccaglia and Luvoni, 2006). To evaluate the effect of litter size on the accuracy, data were grouped for small (<5 pups), normal (5-9 pups) and large (>9 pups) litters in large and giant bitches (Beccaglia *et al.*, 2008). Moreover, predictions within ±1 day and ±2 days were analyzed on the basis of fetal sex ratio in terms of numerical prevalence (≥ 2) of one gender.

Statistical analysis: data were analyzed by Chi-Square test and the level of significance was set at $p<0.05$.

RESULTS

Growth curves of ICC and BP in large and giant size bitches.

The regression analysis resulted in a significant relationship between days before parturition and ICC or BP ($p<0.001$). The derived equations for the prediction of parturition day were:

in large size bitches ICC: $y = (x-105.1)/2.5$ ($r^2=0.92$), and BP: $y = (x-30)/0.8$ ($r^2=0.99$);

in giant size bitches ICC: $y = (x-88.1)/1.9$ ($r^2=0.97$), and BP: $y = (x-29)/0.7$ ($r^2=0.97$).

Accuracy of the ICC and BP for the prediction of parturition day in large and giant size bitches.

The overall accuracy ±2 days of both parameters was significantly higher ($p<0.05$), than the accuracy ±1 day (Table 1). In giant bitches, the accuracy of ICC (±1 day and ±2 days) was significantly more accurate than that of BP.

With regard to litter size, no differences ($p>0.05$) were observed in large bitches for both parameters (Table 2). In giant bitches, only the accuracy of the prediction by BP was significantly lower ($p<0.05$) in small, than normal litter size (Table 3).

No effect of fetal sex ratio was observed on the accuracy (±1 day and ±2 days) of ICC and BP (Table 4, 5).

DISCUSSION

In bitches, a highly accurate prediction of the parturition day is crucial for the proper breeding management. The lack of specific fetal growth curves in large and giant dogs, prompted this investigation. Data showed that ICC and BP are reliable indicators of the gestational age, as proved by the coefficients of determination (r^2) greater than 0.9.

The overall accuracy of the prediction (±1 day and ±2 days) that ranged between 62% and 89% is comparable with the accuracy previously obtained in small and medium dogs by size-related growth curves (Beccaglia and Luvoni 2006). The application of specific formulas for giant dogs increased the BP accuracy compared to what reported in the literature by using non-specific curves (Socha and Janowski 2014).

The accuracy at ±2 days of both parameters was significantly higher than that at ±1 day. This result was foreseeable as, with the extension of the time range, there is an increase of the probability that the actual and the predicted dates will fall within the same time range. Anyhow, 2 days between actual and predicted parturition term might be considered safe and acceptable in clinical practice.

In large bitches, as in small and medium size dogs (Beccaglia and Luvoni 2006), both ICC and BP were equally reliable for the prediction of the delivery day, whereas in giant dogs the prediction should be preferably performed by ICC whose accuracy (±1 day and ±2 days) was significantly higher than that of BP.

This result differs from what has been observed in the aforementioned study in which no differences were found in the accuracy of these parameters in large and giant dogs when formulas for medium dogs were applied to a small number of observations (Socha and Janowski 2014).

The inner chorion cavity may be less affected than BP by individual variability of fetal growth during late gestation (Son *et al*. 2001; Kutzler *et al.* 2003; Beccaglia and Luvoni 2006; Lopate 2008; Socha and Janowski 2014) and this could also explain the effect of litter size on the accuracy of BP measurements.

In small litters of giant dogs, the lower BP accuracy might have been related to the fetuses overgrowth and/or to the consequent gestation length prolongation (Gavrilovic *et al.* 2008). Litter size and duration of gestation are negatively correlated (Okkens *et al.* 1993; Okkens *et al.* 2001) and in giant dogs, where normal numbers of fetuses is generally higher than in large dogs (Sverdrup Borge *et al.* 2011), the effect of the presence of few fetuses could be more evident.

As previously observed in other sizes dogs (Beccaglia and Luvoni 2006), the gender did not affect the accuracy of the prediction and this result is further confirmed by the observation that neonatal sex does not influence newborn bodyweight (Alonge *et al.* 2014; Groppetti *et al.* 2015). In clinical practice, when there is a history of dystocia, the elective cesarean section should be planned. A prediction of the term as much accurate as possible is needed to identify the proper time of surgery and to prevent neonatal loss due to fetal immaturity caused by a pre-term cesarean section. In case of mismating or when the maternal health is threatened, the evaluation of gestational age is also required to apply the more safe and efficient protocol for pregnancy interruption.

A highly accurate prediction of the gestational term might be obtained by a careful estrus monitoring, but often the information about estrous cycle are unavailable and the ultrasonographic fetal biometry is a valid alternative. The enormous variety in size among different canine breeds prompts the use of specific size-related formulas which ensure an accurate identification of the gestational age.

REFERENCES

Alonge, S., Meloni, T., Melandri, M., *et al.* (2014) Factors influencing birth weight and neonatal weight gain in Chow Chow puppies. Proceedings of the European Veterinary Society for Small Animal Reproduction. September 26, Wroclaw, Poland. p 164

Arbeiter, K. (1993) Anovulatory ovarian cycles in dogs. *Journal of Reproduction and Fertility* 47S, 453-456

Beccaglia, M., Luvoni, G.C. (2006) Comparison of the accuracy of two ultrasonographic measurements in predicting the parturition date in the bitch. *Journal of Small Animal Practice* 47, 670–673

Beccaglia, M., Faustini, M., Luvoni, G.C. (2008) Ultrasonographic study of deep portion of diencephalo-telencephalic vesicle for the determination of gestational age of the canine fetus. *Reproduction in Domestic Animals* 43, 367-370

England, G.C.W., Allen, W.E., Porter, D.J. (1990) Studies on canine pregnancy using B-mode ultrasound: develompent of the conceptus and determination of gestational age. *Journal of Small Animal Practice* 31, 324–329

Gavrilovic, B.B., Andersson, K., Linde Forsberg, C. (2008) Reproductive patterns in the domestic dog--a retrospective study of the Drever breed. *Theriogenology* 70, 783-794

Groppetti, D., Ravasio, G., Bronzo, V., *et al.* (2015) The role of birth weight on litter size and mortality within 24h of life in purebred dogs: What aspects are involved? *Animal Reproduction Science* 163, 112-119.

Kim, Y., Travis, A.J., Meyers-Wallen, W.N. (2007) Parturition prediction and timing of canine pregnancy. *Theriogenology* 68, 1177–1182

Kutzler, M.A., Yeager, A.E., Mohammed, H.O., *et al.* (2003) Accuracy of canine parturition date prediction using fetal measurement obtained by ultrasonography. *Theriogenology* 60, 1309–1317

Lévy, X., Fontbonne, A. (2007) Determining the optimal time of mating in bitches: particularities. *Revista Brasileira de Reproduo Animal* 31, 128-134

Lopate, C. (2008) Estimation of gestational age and assessment of canine fetal maturation using radiology and ultrasonography: a review. *Theriogenology* 70, 397–402

Luvoni, G.C., Beccaglia, M. (2006) The prediction of parturition date in canine pregnancy. *Reproduction in Domestic Animals* 41, 27–32

Luvoni, G.C., Grioni, A. (2000) Determination of gestational age in medium and small size bitches using ultrasonographic fetal measurements. *Journal of Small Animal Practice* 41, 292–294

Michel, E., Spörri, M., Ohlerth, S., *et al.* (2011) Prediction of parturition date in the bitch and queen. *Reproduction in Domestic Animals* 46, 926–932

Okkens, A., Hekerman, T., de Vogel, J., *et al.* (1993) Influence of litter size and breed on variation in length of gestation in the dog. *The Veterinary Quarterly 15,160-161*

Okkens, A., Tenissen, J., Van Osch, W., *et al.* (2001) Influence of litter size and breed on the duration of gestation in dogs. *Journal of Reproduction and Fertility* 57s, 193-197

Socha, P., Janowski, T. (2014) Predicting the parturition date in bitches of different body weight by ultrasonographic measurements of inner chorionic cavity diameter and biparietal diameter. *Reproduction in Domestic Animals* 49, 292–296

Socha, P., Janowski, T., Bancerz-Kisiel, A. (2015) Ultrasonographic fetometry formulas of inner chorionic cavity diameter and biparietal diameter for medium-sized dogs can be used in giant breeds. *Theriogenology* 84, 779–783

Son, C., Jeong, K., Kim, J., *et al.* (2001) Establishment of prediction table of parturition day with ultrasonography in small pet dogs. *The Journal of Veterinary Medical Science* 7, 715–721

Sverdrup Borge, K., Tønnessen, R., Nødtvedt, A., *et al.* (2011) Litter size at birth in purebred dogs—A retrospective study of 224 breeds. *Theriogenology* 75, 911–919

Tønnessen, R., Sverdrup Borge, K., Nødtvedt, A., *et al.* (2012) Canine perinatal mortality: A cohort study of 224 breeds. *Theriogenology* 77, 1788–1801

Yeager, A.E., Mohammed, H.O., Meyers-Wallen, V., *et al.* (1992) Ultrasonographic appearance of the uterus, placenta, fetus and fetal membranes throughout accurately timed pregnancy in Beagles. *American Journal of Veterinary Research* 3, 342–351

TABLES

Table 1. Accuracy of parturition day prediction in large and giant size bitches.

Bitch size	±1 day		±2 days	
	ICC diameter, n (%)	BP diameter, n (%)	ICC diameter, n (%)	BP diameter, n (%)
Large	42/65 (64.6)[a]	64/102 (62.8)[a]	54/65 (83.1)[b]	90/102(88.3)[b]
Giant	31/39 (79.5)[A]	31/52 (59.6)[B]	39/39 (100)[C]	44/52 (84.6)[D]
Overall	*73/104 (70.2)*[*]	*95/154 (61.7)*[*]	*93/104 (89.4)*[**]	*134/154 (87)*[**]

ICC Inner chorionic cavity; BP Biparietal
Different superscripts denote significant differences within rows ($p<0.05$).

Table 2. Accuracy of parturition day prediction on the basis of litter size in large bitches.

Litter size	±1 day		±2 days	
	ICC diameter, n (%)	BP diameter, n (%)	ICC diameter, n (%)	BP diameter, n (%)
Large (>9)	6/9 (66.7)	12/23(52.2)	9/9 (100)	20/23 (87)
Normal (5-9)	30/47 (63.8)	45/64 (70.3)	38/47 (80.9)	58/64 (90.6)
Small (<5)	6/9 (66.7)	7/15 (46.7)	7/9 (77.8)	12/15 (80)

ICC Inner chorionic cavity, BP Biparietal.
No differences within columns.

Table 3. Accuracy of parturition day prediction on the basis of litter size in giant bitches.

Litter size	±1 day		±2 days	
	ICC diameter, n (%)	BP diameter, n (%)	ICC diameter, n (%)	BP diameter, n (%)
Large (>9)	9/13 (69.2)	5/9 (55.6)[a]	13/13 (100)	7/9 (77.8)[a]
Normal (5-9)	10/12 (83.3)	22/28 (78.6)[a]	12/12 (100)	27/28 (96.43)[a]
Small (<5)	12/14 (85.7)	4/15 (26.7)[b]	14/14 (100)	9/15 (60)[b]

ICC Inner chorionic cavity, BP Biparietal.
Different superscripts denote significant differences within columns ($p<0.05$).

Table 4. Accuracy of parturition day prediction on the basis of sex ratio in large bitches.

Sex ratio	±1 day		±2 days	
	ICC diameter, n (%)	BP diameter, n (%)	ICC diameter, n (%)	BP diameter, n (%)
>Males	11/18 (61.1)	19/33 (57.6)	13/18 (72.2)	29/33 (87.9)
Males=Females	24/37 (64.9)	25/38 (65.7)	31/37 (83.8)	37/39 (94.9)
>Females	7/10 (70)	20/30 (66.7)	10/10 (100)	24/30 (77.8)

ICC Inner chorionic cavity, BP Biparietal.
No significant differences within columns.

Table 5. Accuracy of parturition day prediction on the basis of sex ratio in giant bitches.

Sex ratio	±1 day		±2 days	
	ICC diameter, n (%)	BP diameter, n (%)	ICC diameter, n (%)	BP diameter, n (%)
>Males	7/9 (77.8)	6/12 (50)	9/9 (100)	10/12 (83.3)
Males=Females	18/22 (81.8)	15/25 (60)	22/22 (100)	21/25 (84)
>Females	6/8 (75)	10/15 (66.7)	8/8 (100)	13/15 (86.7)

ICC Inner chorionic cavity, BP Biparietal.
No differences within columns.

PAPER 2

FETO-MATERNAL HEART RATE RATIO

IN PREGNANT BITCHES:

EFFECT OF GESTATIONAL AGE AND MATERNAL SIZE

(submitted to Reproduction in Domestic Animals)

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FETO-MATERNAL HEART RATE RATIO IN PREGNANT BITCHES: EFFECT OF GESTATIONAL AGE AND MATERNAL SIZE.

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ABSTRACT

Few information are available on parameters that can be used to objectively assess the fetal health during canine pregnancy. To identify a reliable parameter for the evaluation of fetal wellbeing, the effect of pre-gestational maternal bodyweight and gestational age on fetal heart rate (FHR) and on feto-maternal heart rate ratio (FHR/MHR) was investigated. Seventeen client-owned pregnant bitches of different pre-gestational maternal bodyweight were examined by serial echo color Doppler. Only data from 11 uncomplicated pregnancies were included in the statistical analysis. The relationship between FHR, and FHR/MHR, and independent variables was analyzed by polynomial regression ($p \leqslant 0.05$). The FHR and the FHR/MHR significantly fitted a multiple quadratic regression for all independent variables. They both increased from 35 to 20 days before parturition, then a decreasing pattern followed. Higher values of both parameters were observed in bitches of lowest and highest bodyweight.

Patterns of FHR and FHR/MHR were similar, but the ratio better describes the effect of the independent variables on the data. Thus, the highest significance of FHR/MHR compared to FHR alone, encourages the application of this ratio to evaluate fetal wellbeing. The equation derived by the regression analysis of FHR/MHR could be applied in clinical practice to obtain its expected values in healthy pregnancies.

ABRIDGED TITLE

Feto-maternal heart rate ratio in canine pregnancy.

KEY WORDS

dog, pregnancy, heart rate, fetal health

INTRODUCTION

The main goal of a physical examination of a pregnant bitch is the evaluation of fetal development and fetal and maternal wellbeing.

The fetal development in the different gestational ages has been deeply investigated in dogs, but only few information are available on parameters that can be used to objectively assess the fetal health (Johnston et al. 2001). Among these, the fetal heart rate (FHR), recorded by echo color Doppler, is commonly used to monitor viability and to identify distress of the conceptus from 23-25 days after the LH surge until parturition (Yeager and Concannon 1992; Verstegen et al. 1993; Zone and Wanke 2001; Gil et al. 2014).

It has been reported that the normal FHR is 180-220 bpm (Smith 2011), whereas the maternal heart rate (MHR) ranges between 70 and 120 bpm (Lucio et al. 2009). In case of fetal distress a bradycardia is commonly detectable, but the relationship between FHR and MHR has been poorly investigated[1]. Several factors such as pregnancy, maternal age, breed, bodyweight and temperament could affect the MHR (Brooks and Keil 1994a,1994b; Bodey and Michell 1996; Lucio et al. 2009; Hezzell et al. 2013).

The availability of reference values of the ratio FHR/MHR, could better contribute to the evaluation of the fetal health at different gestational ages, than the single FHR values. For this purpose, the trend of FHR and FHR/MHR ratio in bitches of different pre-gestational bodyweight was evaluated during pregnancy.

MATERIALS AND METHODS

Seventeen client-owned pregnant bitches of different breeds (Shih-tzu, Shetland, Jack Russell Terrier, Weimaraner, Boxer, and Great Dane), and pre-gestational bodyweights (5.8-68 kg) aged between 2 and 7 years presented to the Department for breeding management and pregnancy evaluation, were included in this study. Informed owner consent was obtained.

All bitches were healthy at the physical examination and with no history and signs of cardiac diseases. For breeding management, the day of the ovulation was considered to be when plasma progesterone concentrations ranged between 4-10 ng/ml (Arbeiter 1993; Levy and Fontbonne 2007), as evaluated using an Enzyme Linked Fluorescent Assay[1].

According to owner's availability, ultrasound examinations were performed in five bitches twice a week from day 21 after ovulation, and in 12 bitches at week 4, 7, and 9 of pregnancy.

Two-dimensional, gray-scale, real-time ultrasound and echo color Doppler images were produced using a 7.5 MHz microconvex probe[2]. The bitches were positioned in lateral recumbency, transmission gel was applied and MHR was evaluated at the level of the aortic valve for three times (at the beginning of the examination, after 10 minutes and at the end of the examination) to reduce and control the stress-effect induced by the restraint. Fetuses' normal development was assessed by the measurement of ultrasonographic extra-fetal and fetal parameters (Luvoni and Grioni 2000; Alonge et al. 2015).

The FHR values of at least three different fetuses (in litter size $\geq$3) were recorded in each examination.

The day of parturition and the neonatal survival were reported by the owners. Only data from uncomplicated pregnancies with no evidence of embryo, fetal or neonatal loss were included in the statistical analysis.

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Statistical analysis

A polynomial regression model was adopted to analyze the relationship between FHR, MHR, FHR/MHR ratio and independent variables (pre-gestational maternal bodyweight and gestational age, in terms of days from parturition). Statistical significance was set at $p \leq 0.05$.

RESULTS

Results include eleven uncomplicated pregnancies of bitches of different pre-gestational bodyweight (5.8-68 kg).

The FHR values fitted a multiple quadratic regression (saddle, Fig. 1), with significance at $p \leq 0.02$ of all independent variables. Multiple r was about 0.50, a mean value, with a low determination coefficient ($r^2 = 0.25$). The coefficients of the regression, the significance, and the confidence intervals are reported in Table 1. An increase of FHR was observed from 35 to 20 days before parturition. After the maximum, the curve followed a decreasing pattern until parturition. Higher values of FHR were observed in bitches of lowest and highest bodyweight.

Data of MHR fitted significantly a linear regression equation ($p<0.01$ for all parameters, Fig. 2). Multiple r was 0.66, and the multiple $r^2=0.43$. The coefficients of the regression, the significance, and the confidence intervals are reported in Table 2. An increase of MHR was observed close to term and in bitches of high bodyweight.

The ratio of FHR/MHR well fitted a multiple quadratic regression (saddle, Fig. 3), with all independent variables with significance at $p<0.05$. Multiple r was 0.71, with r^2 0.50.

Therefore, FHR/MHR ratio was well expressed by a quadratic law, in terms of pre-gestational maternal bodyweight and gestational age. The coefficients of the regression, the significance, and the confidence intervals are reported in Table 3. As seen for FHR, the FHR/MHR ratio resulted higher in low and high bodyweight, and it reached the maximum values at about 20 days before parturition.

The equation derived from the quadratic regression was as follows:

$z = 1.8284 - 0.0137x + 0.00014x^2 + 0.05071y - 0.00099y^2$,

where z = FHR/MHR ratio, x = pre-gestational maternal bodyweight (kg), y= days before parturition.

DISCUSSION

The FHR is a good indicator of fetal wellbeing, but its regulatory mechanisms and variability along pregnancy are still poorly understood. As previously mentioned, fetal distress is the main cause of FHR alteration, but other factors such as gestational age and pre-gestational maternal bodyweight should be taken into account.

Present results demonstrate that in all bitches the FHR increased during pregnancy until 20 days before parturition and then a reduction was observed toward the term. These results are in agreement with those previously reported in an experimental colony of Beagles where an increase from 214+/-13.3 to 238.2+/-16.1 bpm at day 40 of pregnancy and a decrease to 218+/-6.7 bpm close to term were observed (Verstegen et al. 1993).

Such condition could be explained by the dynamics of the circulatory system maturation. Both in human and in veterinary medicine FHR trend is mainly related to the autonomic nervous system development and activity (Verdurmen et al. 2013). Sympathetic and parasympathetic control of circulatory functions mature at different rates during fetal development and the former becomes active earlier in fetal life, than the latter (Assali et al. 1977; Woods et al. 1977). For this reason when the FHR is mainly under the sympathetic effect, its values are higher than at the end of pregnancy, when the development of the parasympathetic system starts to occur.

Even though the FHR trend is similar in dogs of different size, in low and high bodyweight bitches, the values were higher. In small dogs this finding might be related to their physiological higher sympathetic tone, whereas it was unexpected in heavy dogs, characterized by a prevalence of the parasympathetic tone (Hezzell et al. 2013). It could be hypothesized that in these dogs the immaturity of parasympathetic system and the early development of the sympathetic system might be responsible of the higher FHR.

Concerning MHR, present results demonstrated an increase during pregnancy likely to provide adequate blood supply to the fetus, as already reported in the literature (Lucio et al. 2009).

The patterns of FHR and FHR/MHR ratio were similar, but the ratio better described the effect of the independent variables, such as maternal bodyweight and gestational age, on the data ($r^2 = 0.50$ vs. $r^2 = 0.25$). The non-linear trend of the ratio, (i.e. increase until 20 days before parturition followed by a remarkable decrease) suggests that in the late pregnancy the fetus may be able to manage, at least partially, maternal cardiovascular fluctuations, as observed in women and ewes. In this period MHR increased linearly, whereas FHR and the ratio decreased toward the term to ensure the best subsistence of the conceptus (Eisenberg de Smoler et al. 1975; Bocking et al. 1985; Van Leeuwen et al. 2003, 2009).

Among factors that affect FHR and FHR/MHR ratio, fetal movements should be considered. They have been deeply investigated in women and large animals (Dawes et al. 1992; Di Renzo et al. 1994; Baska-Vincze et al. 2014), in which changes in FHR according to normal rates of movements have been described. Few information are available in dogs in which temporary accelerations of FHR may be associated with fetal movements (Davidson 2003), but normal rates of fetal activity during different pregnancy periods, have not yet been defined. Therefore, a potential effect of fetal movements on FHR deserves further investigations in this species. It remains also to evaluate the potential effect of the litter size on FHR and on FHR/MHR ratio.

In clinical practice, for the proper management of pregnancy, an accurate assessment of fetal viability and health is crucial to achieve an early diagnosis of complications.

In puppies, a neonatal loss less than 8% is considered acceptable (Tønnessen et al. 2012). However, to not negatively affect the results of this study, only data from pregnancies with no evidence of embryo, fetal or neonatal loss were included in the data analysis to prevent to record any abnormal FHR.

This strict recruitment ensures a satisfying sensibility and specificity of the results that suggest that the maternal pre-gestational bodyweight and the gestational age influence both FHR and FHR/MHR ratio. The highest significance of FHR/MHR ratio, compared to FHR, encourages the application of this ratio to evaluate fetal wellbeing. For this reason the obtained equation for FHR/MHR ratio, that describes the trend in healthy fetuses, could be helpful in clinical practice to derive expected values in uncomplicated pregnancies.

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.

AUTHORS CONTRIBUTION

SA, MM, MF and GCL contributed to design the study, collect and analyze the data, and draft the paper. All authors have approved the final version.

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REFERENCES

Alonge S, Beccaglia M, Melandri M, Luvoni GC, 2015: Prediction of parturition term in large and giant size bitches by ultrasonographic measurements. In: Schäfer-Somi S, Podhajsky E, Günzel-Apel AR, Hagman R (eds.), Proc 18th Ann Congr EVSSAR, Hannover (Germany), 11-12 September 2015, p. 145.

Arbeiter K, 1993: Anovulatory ovarian cycles in dogs. J Reprod Fertil 47 Suppl, 453-456.

Assali NS, Brinkman CR, Woods JR, Dandavino A, Nuwayhid B, 1977: Development of neurohumoral control of fetal, neonatal, and adult cardiovascular functions. Am J Obstet Gynecol 129, 748-759.

Baska-Vincze B, Baska F, Szenci O, 2014: Transabdominal ultrasonographic evaluation of fetal well-being in the late-term mare and cow. Acta Vet Hung 62, 439-451.

Bocking AD, Harding R, Wickham PJ, 1985: Relationship between accelerations and deceleration in heart rate and skeletal muscle activity in sheep. J Dev Physiol 7, 47-54.

Bodey AR, Michell AR, 1996: Epidemiological study of blood pressure in domestic dogs. J Small Anim Pract 37, 116-125.

Brooks VL, Keil LC, 1994a: Hemorrage decreases arterial pressure sooner in pregnant compared with nonpregnant dogs: role of baroreflex. Am J Physiol 266, 1610-1619.

Brooks VL, Keil LC, 1994b: Changes in the baroreflex during pregnancy in conscious dogs: heart rate and hormonal responses. Endocrinology 135, 1894-1901.

Davidson A, 2003: Obstetrical monitoring in dogs. Vet Med 6, 508-516.

Dawes GS, Moulden M, Redman CW, 1992: Short-term fetal heart rate variation, decelerations, and umbilical flow velocity waveforms before labor. Obstet Gynecol 80, 673-678.

Di Renzo GC, Copray FJA, O'Herlihy C, Van Geijn HP, 1994: Maternal-fetal surveillance within the European community. In: Van Gejin HP, Copray FA (eds), A critical appraisal of fetal surveillance. Elsevier Science Amsterdam, pp. 11-15.

Eisenberg de Smoler P, Smith HC, Karchmer S, 1975: Correlation of fetal heart rate, maternal heart rate, and age of pregnancy. Am J Obstet Gynecol 121, 62-65.

Gil E.M., Garcia D.A., Giannico A.T., et al. (2014) Canine fetal heart rate: do accelerations or decelerations predict the parturition day in bitches? Theriogenology. 82, 933-941.

Hezzell MJ, Humm K, Dennis SG, Agee L, Boswood A, 2013: Relationship between heart rate and age, bodyweight and breed in 10849 dogs. J Small Anim Pract 54, 318-324.

Johnston SD, Root Kustritz MV, Olson PNS, 2001: Canine pregnancy. In: Johnston SD, Root Kustritz MV, Olson PNS (eds), Canine and feline theriogenology. WB Saunders Co Philadelphia, pp. 66-104.

Lévy X, Fontbonne A, 2007: Determining the optimal time of mating in bitches: particularities. Rev Bras Reprod Anim 31, 128-134.

Lucio CF, Silva LCG, Rodrigues JA, Veiga GAL, Vannucchi CI, 2009: Peripartum haemodynamic status of bitches with normal birth or dystocia. Reprod Domest Anim 44, 133-136.

Luvoni GC, Grioni A, 2000: Determination of gestational age in medium and small size bitches using ultrasonographic fetal measurements. J Small Anim Pract 41, 292-294.

Smith FO, 2011: Prenatal care of the bitch and queen. In: Peterson ME, Kutzler MA (eds), Small animal pediatrics. Elsevier Saunders St. Louis, pp. 1-10.

Tønnessen R, Borge KS, Nødtvedt A, Indrebø A, 2012: Canine perinatal mortality: a cohort study of 224 breeds. Theriogenology 77, 1788-1801.

Van Leeuwen P, Geue D, Lange S, Cysarz D, Bettermann H, Grönemeyer D, 2003: Is there evidence of fetal-maternal heart rate synchronization? BMC Physiol 3, 2.

Van Leeuwen P, Geue D, Thiel M, Cysarz D, Lange S, Romano MC, Wessel N, Kurths J, Grönemeyer D, 2009: Influence of paced maternal breathing on fetal-maternal heart rate coordination. Heart-rate characteristics and effects of maternal respiratory sinus arrhythmia, in Proceedings. Natl Acad Sci 106, 13661-13666.

Verdurmen K, Renckens, J, van Laar J, Oei SG, 2013: The influence of corticosteroids on fetal heart rate variability: a systematic review of the literature. Obstet Gynecol Surv 68, 811-824.

Verstegen JP, Silva LD, Onclin K, Donnay I, 1993: Echocardiographic study of heart rate in dog and cat fetuses in utero. J Reprod Fertil 47 Suppl, 75-80.

Woods JR, Dandavino A, Murayama K, Brinkman CR, Assali NS, 1977: Autonomic control of cardiovascular functions during neonatal development and in adult sheep. Circulation Res 40, 401-407.

Yeager AE, Concannon PW, 1990: Association between the preovulatory luteinizing hormone surge and the early ultrasonographic detection of pregnancy and fetal heartbeats in Beagle dogs. Theriogenology 34, 655-665.

Zone MA, Wanke MM, 2001: Diagnosis of canine fetal health by ultrasonography. J Reprod Fertil 57 Suppl, 215-219.

FIGURE TITLES

Figure 1. Quadratic regression for fetal heart rate (FHR) in bitches as a function of different pre-gestational bodyweight at different gestational age (days from parturition).

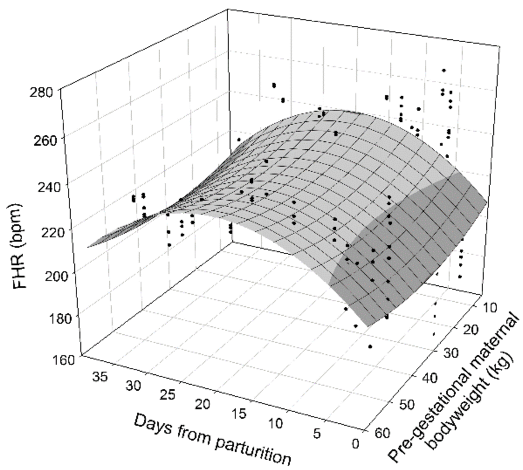

Figure 2. Linear regression for maternal heart rate (MHR) in bitches as a function of pre-gestational bodyweight at different gestational age (days from parturition).

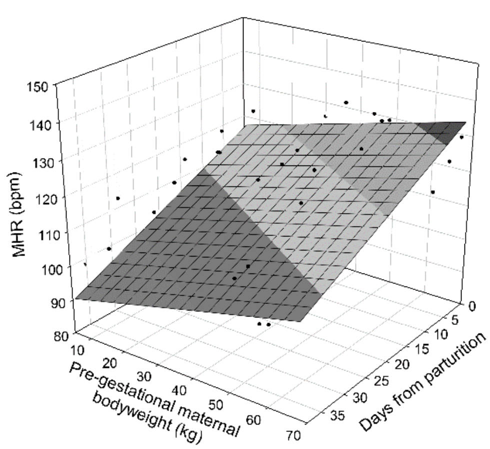

Figure 3. Polynomial quadratic regression for feto-maternal heart rate (FHR/MHR) ratio in bitches of different pre-gestational bodyweight at different gestational age (days from parturition).

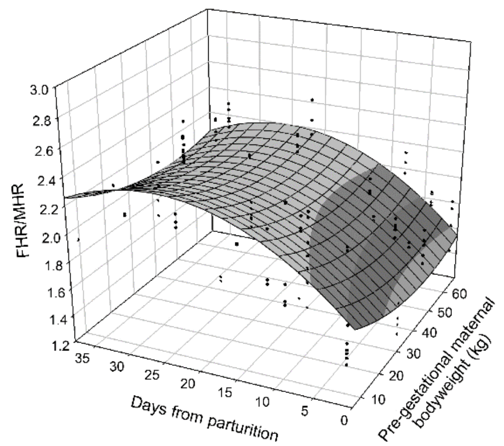

TABLE TITLES

Table 1. Coefficients for the quadratic regression of FHR in bitches of different pre-gestational bodyweight at different gestational age (days from parturition). Significances and 95% confidence intervals are reported.

	FHR coefficient	p	-95%	+95%
Intercept	211.6732	0.0001	202.9648	220.3817
Pre-gestational bodyweight	-0.6935	0.01	-1.2262	-0.1609
Pre-gestational bodyweight ^{2}	0.0100	0.016	0.0019	0.0182
Days from parturition	3.2479	0.0001	2.3645	4.1312
Days from parturition^2	-0.0810	0.0001	-0.1061	-0.0559

Table 2. Coefficients for the linear regression of MHR in bitches of different pre-gestational bodyweight at different gestational age (days from parturition). Significances and 95% confidence intervals are reported.

	MHR coefficient	p	-95%	+95%
Intercept	115.436	0.0001	100.896	130.178
Pre-gestational bodyweight	0.222	0.004	-0.123	0.595
Days from parturition	-0.600	0.001	-1.411	0.059

Table 3. Coefficients for the quadratic regression of FHR/MHR ratio in bitches of different pre-gestational bodyweight at different gestational age (days from parturition). Significances and 95% confidence intervals are reported.

	FHR/MHR coefficient	p	-95%	+95%
Intercept	1.828405	0.0001	1.696833	1.959976
Pre-gestational bodyweight	-0.013705	0.001	-0.021753	-0.005658
Pre-gestational bodyweight ^{2}	0.000141	0.03	0.000017	0.000264
Days from parturition	0.050715	0.0001	0.037369	0.064061
Days from parturition^2	-0.000986	0.0001	-0.001365	-0.000607

OTHER PUBBLICATIONS AND CO-AUTORSHIP

International peer-reviewed journals:

1. **Alonge S.**, Romussi S, Grieco V., Luvoni G.C. (2015) *Congenital abnormality of the vagina complicated by hemato-pyocolpos in a 1-year labrador retriever.* Reproduction in Domestic Animals, 50:514-516

2. Caloni F., Cortinovis C., Rivolta M., **Alonge S.**, Davanzo F. (2013) *Plant poisoning in domestic animals: epidemiological data from an italian survey (2000-2011).* Veterinary Record, 172:580-583

Submitted to International peer-reviewed journals:

- Beccaglia M., **Alonge S.**, Trovò C., Luvoni G.C. Determination of gestational time and prediction *of parturition: An update.* Reproduction in Domestic Animals.

International congress proceedings:

I. **Alonge S.**, Faustini M., Luvoni G.C. (2015) *Fetal and maternal heart rate ratio in pregnant bitches: effect of gestational age and maternal bodyweight*. Proceedings 18th European Veterinary Society Small Animal

Reproduction (EVSSAR) Congress, Hannover, Germany, 11-12 September, p. 122

II. **Alonge S.**, Beccaglia M., Melandri M., Luvoni G.C. (2015) *Prediction of parturition term in large and giant size bitches by ultrasonographic measurements.* Proceedings 18th European Veterinary Society Small Animal Reproduction (EVSSAR) Congress, Hannover, Germany, 11-12 September, P. 145

III. Mantziaras G., **Alonge S.**, Faustini M., Luvoni G.C. (2015) *Aging of the prostate in dogs. Part I: appearance of abnormal ultrasonographic findings.* Proceedings 18th European Veterinary Society Small Animal Reproduction (EVSSAR) Congress, Hannover, Germany, 11-12 September, p. 115

IV. Mantziaras G., **Alonge S.**, Faustini M., Luvoni G.C. (2015) *Aging of the prostate in dogs. Part II: changes of prostatic size in breeds with different longevity.* Proceedings 18th European Veterinary Society Small Animal Reproduction (EVSSAR) Congress, Hannover, Germany, 11-12 September, p. 182

V. Spalla I., **Alonge S.**, Locatelli C., Melandri M., Brambilla P.G., Bussadori M.C. (2015) *Changes in the biomechanics of the left ventricle in healthy young and adult Great Danes.* Proceedings 2015 ACVIM - American College Of Veterinary Internal Medicine - Forum, Indianapolis, USA, 4-6 June, p. 22-23

VI. **Alonge S.**, Meloni T., Melandri M., Faustini M., Luvoni G.C., Veronesi M.C. (2014) *Factors influencing birth weight and neonatal weight gain in Chow-Chow puppies.* Proceedings 17th European Veterinary Society Small

Animal Reproduction (EVSSAR) Congress, Wroclaw, Poland, 26 September, p. 164

VII. **Alonge S.**, Romussi S., Grieco V., Luvoni G.C. (2014) *Congenital abnormality of the vagina complicated by hemato-pyocolpos in a 1-year Labrador Retriever.* Proceedings 17th European Veterinary Society Small Animal Reproduction (EVSSAR) Congress, Wroclaw, Poland, 26 September, p. 158

VIII. Mantziaras G., **Alonge S**., Luvoni G.C. (2014) *Ultrasonographic study of age-related changes on the size of prostate and testicles in healthy German Shepherd dogs*. Proceedings 17th European Veterinary Society Small Animal Reproduction (EVSSAR) Congress, Wroclaw, Poland, 26 September, p. 150

IX. Mantziaras G., **Alonge S.**, Luvoni G.C. (2014) *Effect of hyperadrenocorticism on ultrasonographic findings in prostate and testicles of aged dogs*. Proceedings 17th European Veterinary Society Small Animal Reproduction (EVSSAR) Congress, Wroclaw, Poland, 26 September, p. 194

X. **Alonge S.**, Melandri M., Meloni T., Morselli M.G., Luvoni G.C. (2013) *Could a single p4 determination predict the days for successful breedings in bitches?* Proceedings 16th European Veterinary Society Small Animal Reproduction (EVSSAR) Congress, Toulouse, France, 5-6 July, p 135

XI. Meloni T., **Alonge S.**, Martino P.A., Veronesi M.C. (2013) *Whelping management in a bitch with vaginal ß-haemolytic streptococci.* Proceedings 16th European Veterinary Society Small Animal Reproduction (EVSSAR) Congress, p 154 Toulouse, France, 5-6 July, p. 154