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Effect of xylazine, isoxsuprine and lidocaine on Doppler sonographic uterine and umbilical blood flow measurements in cows during the last month of pregnancy

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Abstract

The maternal portion of the bovine placenta receives blood mainly from the uterine arteries and the fetal portion from the umbilical arteries. Placental perfusion is crucial for fetal development and undergoes adaptive changes during pregnancy according to fetal requirements. One goal of this study was to investigate changes in Doppler sonographic measurements of blood vessels that supply blood to the placenta in cows during the last four weeks of pregnancy. Another goal was to examine how these measurements are affected by three drugs commonly used in cows at the time of parturition. Nine cows underwent Doppler sonographic examination of the uterine arteries (AUT) ipsilateral and contralateral to the pregnant horn and one umbilical artery (AUM) three times per week during the last four weeks of gestation. This was followed by the randomized administration of one of the three following experimental drugs per day: isoxsuprine (200 mg/cow, intravenously), xylazine (2 mg/100 kg, intravenously) and lidocaine for epidural anesthesia (100 mg/cow). Doppler sonographic examination was repeated 30 minutes after medication.

Maternal pulse rate increased during the study period ($P < 0.001$) and the diameter of the contralateral AUT was smaller in the last week before birth than in the two preceding weeks. The resistance index (RI) of the ipsilateral AUT was smaller in the last week than in the first two weeks of the study period. Uterine blood flow

volume (BFV) increased after isoxsuprine by 5% and after epidural anesthesia by 6% (both $P \leq 0.05$) and decreased after xylazine by 10% ($P < 0.001$). Isoxsuprine was the only drug that elevated the BFV in the AUM ($P \leq 0.05$). Xylazine increased the RI of both AUT (both $P < 0.001$) and significantly reduced maternal and fetal pulse frequencies, whereas isoxsuprine significantly reduced the RI of both AUT and the AUM and increased maternal and fetal pulse frequencies. The results showed that Doppler sonographic measurements of uterine and umbilical arteries change little in the last month of pregnancy in the cow. Isoxsuprine and epidural anesthesia with lidocaine have the potential to improve uterine perfusion.

Keywords: Cow; fetus; gestation; ultrasound; Doppler ultrasound; intrauterine resuscitation

1. Introduction

Color Doppler sonography has become the diagnostic technique of choice in human medicine for assessing placental function and fetal wellbeing. This imaging modality has been used routinely for years for monitoring pregnant women and to establish a prognosis in conditions such as fetal growth restriction [1]. Doppler sonography is non-invasive and provides information pertaining to pulse waves, vascular resistance, blood flow volumes and changes in blood flow [2-5]. For example, analysis of blood flow velocity and blood flow resistance in the umbilical arteries (AUM), uterine arteries (AUT) and fetal blood vessels aids in the identification of morphological changes in the fetoplacental vascular bed.

Doppler sonography has also been used to study uterine perfusion in cows during normal pregnancy [6-8]. Because the AUT provide the major portion of blood supply to the bovine uterus, changes in blood flow in these vessels reflect changes in uterine perfusion [9]. The cranial half of the uterus also receives blood from the uterine branch of the ovarian artery [10]. Time-averaged maximum blood flow velocity (TAMV) increased and resistance index (RI), which measures vascular resistance in tissues distal to the point of examination, decreased during pregnancy [6]. Another study showed a massive increase in blood flow volume during the second half of

gestation as a function of the birth weight of the calf [8]. The increase was based mainly on an increase in the diameter of blood vessels rather than on an increase in blood flow velocity.

Changes in fetal blood supply through the umbilical vessels have been investigated in several animal species. There has been one study on umbilical blood flow in calves during parturition using sonographic transducers fixed to the umbilical vessels [11], and several others in canine, equine and ovine fetuses [12-16].

Transabdominal Doppler sonographic examination of an AUM in pony mares at the 5^{th}, 8^{th} and 10^{th} month of pregnancy revealed a progressive decrease in the ratio between systolic and diastolic blood pressure [12]. In contrast, transrectal Doppler sonographic examination of the AUM in pregnant mares starting at week 19 showed an increase in RI in the last few weeks of gestation [13]. In the pregnant bitch, a progressive increase occurred in the time averaged maximum velocity in the AUM and a decrease in RI between the 4^{th} and 9^{th} week of gestation [14].

Toward the end of gestation, both the placenta and fetus undergo profound maturation processes [15]. One goal of this study was therefore to examine the effects of vascular changes in placenta and fetus on Doppler sonographic measurements of the AUT and AUM in the last month of gestation in cows.

In human medicine, a variety of measures are used to determine and prevent fetal hypoxia. These include prepartal and peripartal monitoring of uterine, placental and fetal perfusion but also pharmacological interventions to improve uterine perfusion in pregnant women and women giving birth [16]. These steps are referred to as intrauterine resuscitation and may include the administration of tocolytic agents to control excessive uterine contractions, in cases of fetal growth restriction or chronic asphyxia [17]. To our knowledge, analogous studies investigating the positive and adverse effects of commonly used drugs on uterine perfusion in pregnant cows have not been published. The drugs most commonly used in bovine obstetrics are lidocaine for epidural anesthesia, and isoxsuprine and xylazine. Isoxsuprine, a β_1- and β_2-adrenomimetic drug, is used to relax the uterus to facilitate fetal mutations or to exteriorize the uterus during Caesarean section. Isoxsuprine relaxes the smooth musculature of the uterus and blood vessels and has a peripheral vasodilatory effect [18,19]. It stimulates β_2-receptors to activate adenylyl cyclase, which results in an increase in myometrial cAMP and thus a decrease in uterine contractility. Isoxsuprine also affects phosphodiesterase, which converts cAMP to AMP [20,21].

Xylazine has the opposite effect. A study in pregnant goats showed that it stimulates postsynaptic uterine α_2-receptors causing an increase in uterine tone and a decrease in uterine arterial blood flow [22]. It is used for sedation of fractious animals and also has analgesic and muscle-relaxant properties [23].

Epidural anesthesia with a local anesthetic drug is used mainly during conservative or surgical obstetrical procedures to block the Ferguson reflex and the evacuation reflex. With an appropriate dose of local anesthetic, the anogenital region, the pelvic area and the proximal part of the udder can be desensitized without inducing recumbency [24]. Epidural anesthesia is used in women for pain control during labor [25] and for caesarean sections because it has fewer adverse effects on uterine and fetal perfusion compared with general anesthesia [26]. Lidocaine is a commonly used local anesthetic and has intermediate efficacy and duration of action compared with other similar drugs. It affects the permeability of the sodium and potassium channels in the cell membrane of neurons, thus blocking the action potential [27]. By blocking Na^+ and K^+ channels in the dorsal horn neurons of the spinal cord, it stops the transmission of pain-induced stimuli [28].

It is conceivable that isoxsuprine, xylazine and lidocaine also affect uterine and fetal perfusion in cattle and thus are involved in fetal hypoxia. Another goal of this study was therefore to examine the effects of these drugs on the hemodynamics of uterine and umbilical arteries in cows during the final four weeks of gestation.

2. Materials and Methods

2.1 Animals

Seven Braunvieh and two Red Holstein cows, which ranged in age from 3 to 17 years and weighed between 720 and 866 kg, were used. Parity ranged from second to 14th. The cows were brought to our clinic 4 weeks before the calculated due date. They were kept in tie stalls, bedded with straw and fed hay, grass silage and water ad libitum. They had daily access to pasture. The cows were moved to a straw-bedded pen upon the first signs of calving and remained there until after delivery of the placenta.

The cows underwent daily clinical examination and transrectal manual and sonographic examinations to identify which uterine horn was pregnant and to confirm that the fetus was alive. The sonographic appearance of the amniotic and allantoic fluid was monitored. The calves were examined clinically immediately after birth.

2.2 Study design

The cows were placed in a chute and were allowed to eat and drink during sonographic examinations. Before the first examination, the right lower flank was clipped from the ventral midline to the level of the stifle. The examinations, which lasted from 1.5 to 3 hours, started 30 minutes after clipping. During the 4 weeks before the calculated due date, the cows were examined sonographically every Monday, Wednesday and Friday by the same examiner (DW). First the position of the fetus was determined using B mode sonography via the right flank, and then one AUM was examined via the right flank and both AUT were examined transrectally using B mode and Doppler sonography.

One of the three experimental drugs was then administered. The other two drugs were given on the other two examination days of the same week; a given drug was used only once per cow per week. The order of drug administration varied among cows and changed weekly. The drugs were administered according to the recommendations of the manufacturer. Isoxsuprine (Isoxsuprini hydrochloridum 10 mg/ml, Degraspasmin, Graeub, Bern, Switzerland) was administered into a jugular vein at a dose of 200 mg/cow. Xylazine (20 mg/ml, Xylazin Streuli, Streuli Pharma, Uznach, Switzerland) was given into a coccygeal vene at the tail site at a dose of 0.02 mg/kg. Epidural anesthesia was achieved with 5 ml 2% lidocaine (Lidocain 20 mg/ml, Lidocain 2% Chassot, Vétoquinol, Ittingen, Switzerland) injected into the sacrococcygeal space or between the first two coccygeal vertebrae. B mode and Doppler sonographic examination of both AUT and one AUM was carried out 30 minutes after medication.

2.3. B mode and Doppler sonography

A LOGIQ e ultrasound machine (GE Medical Systems, Glattbrugg, Switzerland) with a 3.5 MHz convex transducer for transabdominal examinations of the umbilical cord and a 10 MHz linear transducer for transrectal examinations of the AUT was used. Using B mode sonography, the umbilical cord was usually identified

near the uterine wall and one of the AUM was imaged transabdominally in the right ventral flank cranial to the udder. The typical triad of two arteries and two veins confirmed the identity of the cord, and color Doppler sonography was used to identify the direction of blood flow. Three to 5 transverse images of the umbilical cord were taken in B mode for measuring the arterial diameter and 5 to 15 Doppler sonographic images were taken for blood flow analysis; all images were saved for further analysis.

Both AUT were examined transrectally and were identified as described [29]. The external iliac arteries were identified at the point where they branched off the aorta. Following the external iliac artery ventrally, the AUT was identified crossing the external iliac artery. To confirm the identity of the AUT, the artery was followed dorsally toward the internal iliac artery, during which the *ligamentum teres vesicae* was encountered. This is the atrophied stump of the umbilical artery and it allowed the final identification of the AUT. The AUT was then traced back toward the uterus and the point of measurement located immediately cranial to the point where the AUT crossed the external iliac artery and vein. Three to 5 transverse images of both AUT in B mode and 1 to 3 Doppler sonographic images were taken. Measurements were repeated 5 to 8 times. For this purpose, the transducer was temporarily removed from the site of measurement and repositioned after a period of at least 30 seconds to carry out the next measurement.

For generation of optimum spectral displays, an insonation angle of 20° to 60° was chosen for the AUT and AUM. The AUT was first imaged using color Doppler sonography to set the angle, and then spectral graphs were generated in spectral mode. For maximum resolution the spectrum was adjusted to the magnitude of the pulse wave. The images were saved on the ultrasound machine and then uploaded to a personal computer.

2.4. Data collection, analysis and statistics

The images were visually assessed using Photoshop Album Starter Edition 3.0 (Adobe, Zürich, Switzerland) and selected for analysis. Three sharp gray-scale images of arteries with the most circular cross-section were selected and the arterial diameter (DM) was measured and the mean calculated. Four spectral displays with regular representative waveforms were selected and analyzed using PixelFlux Scientific (Chameleon Software, Freiburg i. Br., Germany). The time-averaged

maximum velocity (TAMV) and RI and their means were calculated for 4 cardiac cycles. The maximum velocity of the arterial blood during a cardiac cycle is derived from the formula TAMV [cm/s] = (TAMF x c) / (2F x cos α) where TAMF represents the time-averaged maximum rate shift over the cardiac cycle, c the ultrasound propagation speed, F the transmitted wave rate, and α the angle between the ultrasound beam and the blood flow direction.

The RI was used as an expression of the resistance in the vascular bed distal to the point of examination and calculated using the formula RI = (D-S)/S, where D is the end-diastolic blood flow velocity and S the peak-systolic velocity. The blood flow volume (BFV) was calculated using the formula BFV [cm^3/sec] = TAMV * A, where A is the arterial circumference, derived from the DM and using $A = \pi * (DM/2)^2$. The pulse rate (PR) was defined as number of spectral waves per unit of time [min^{-1}].

The program StatView 5.0 (SAS Institute, Wangen, Switzerland) was used for statistical calculations. For each variable, means and standard deviations were calculated before and after the administration of each drug. Changes in the measured variables during the study period were analyzed using ANOVA for repeated measures. Fisher's PLSD test was used to analyze differences among measurements obtained in different weeks. Differences between measurements before and after the administration were analyzed using a paired *t*-test. For comparison among drugs, differences in measurements obtained after the administration of different drugs were also analyzed using a paired *t*-test. A *P*-value ≤ 0.05 was considered significant.

2.5 Animal experiment permission

This study was authorized by the veterinary office of the Canton of Zurich (permit number 227-2008).

3. Results

All cows calved spontaneously or supported by mild manual traction after a mean gestation length of 286± 2 days. There were 5 right-horn and 4 left-horn pregnancies and 4 heifer and 5 bull calves.

3.1. Changes in B mode and Doppler sonographic variables during the last 4 weeks of gestation

3.1.1. Pulse rate in cows and fetuses

The PR of the dam, assessed in both AUT, increased significantly during the study period (both $P< 0.001$). The PR of the ipsilateral AUT increased from 78.9 min^{-1} to 84.9 min^{-1}.The changes in pulse rate in the AUM were not significant (Fig. 1).

3.1.2. Diameter of the AUT and AUM

The diameter of the AUM and ipsilateral and contralateral AUT did not change over the study period but there were differences between weekly means for the contralateral AUT; the DM in the last week before birth (10.30 ± 1.55 mm) was smaller than in week -3 (10.61 ± 1.71 mm) and in week -2 (10.63 ± 1.63 mm; both $P \leq 0.05$).

3.1.3. Resistance index, blood flow velocity and blood flow volume in the AUT and AUM

The changes in RI of the AUT and AUM over the study period were not significant. In the ipsilateral AUT, the RI ranged from 0.472 ± 0.059 to 0.513 ± 0.042, in the contralateral AUT from 0.566 ± 0.085 to 0.590 ± 0.068 and in the AUM from 0.595 ± 0.087 to 0.620 ± 0.052. The RI of the ipsilateral AUT was significantly greater in week -4 (0.513 ± 0.042) and -3 (0.508 ± 0.052) of the study period than in the last week (0.472 ± 0.059; $P \leq 0.05$).

The TAMV and mean BFV of the arteries did not change significantly during the study period.

3.2. Comparison of B mode and Doppler sonographic measurements before and after medication

253 ***3.2.1. Pulse rates in cows and fetuses***

254 The maternal PR, assessed in both AUT, increased after isoxsuprine from
255 80.8 ± 6.5 min^{-1} to 132.0 ± 10.5 min^{-1} and decreased after xylazine from 79.8 ± 6.8
256 min^{-1} to 66.0 ± 5.9 min^{-1} (Figs. 2a, b; both $P < 0.0001$). There was a trend for a
257 difference between maternal PR in the ipsilateral AUT before and after epidural
258 anesthesia ($P = 0.06$), and a significant difference for the contralateral AUT
259 (decrease from 81.4 ± 6.7 min^{-1} to 79.7 ± 6.4 min^{-1}; Fig. 2b; $P < 0.01$). The pulse rate
260 in the AUM increased after isoxsuprine from 119.1 ± 14.5 min^{-1} to 163.1 ± 23.6 min^{-1}
261 and decreased after xylazine from 117.0 ± 13.5 min^{-1} to 110.5 ± 9.8 min^{-1} (Fig. 2c;
262 both $P < 0.001$).

263 ***3.2.2. Diameter of the AUT and AUM***

264 The DM of the ipsilateral AUT, but not the contralateral AUT, decreased after
265 epidural anesthesia from 14.1 ± 1.1 mm to 13.9 ± 1.3 mm (Fig. 3a; $P < 0.001$). The
266 DM of the contralateral AUT increased after isoxsuprine from 9.9 ± 1.8 mm to 10.9 ±
267 2.0 mm (Fig. 3b; $P < 0.001$) and also after xylazine from 9.8 ± 2.1 mm to 10.2 ± 2.0
268 mm (Fig. 3b; $P < 0.001$). The DM of the AUM was not affected by any of the drugs.

269 ***3.2.3. Resistance index of the AUT and AUM***

270 The RI of the ipsilateral and contralateral AUT decreased after isoxsuprine
271 from 0.484 ± 0.050 to 0.387 ± 0.040 and from 0.561 ± 0.058 to 0.498 ± 0.079,
272 respectively (both $P < 0.001$) and increased after xylazine from 0.496 ± 0.057 to
273 0.558 ± 0.074 and from 0.585 ± 0.073 to 0.645 ± 0.080, respectively (Figs. 4a, b;
274 both $P < 0.001$). The RI of the AUM decreased significantly after isoxsuprine from
275 0.611 ± 0.077 to 0.481 ± 0.080 (Fig. 4c; $P < 0.001$). The RI of the examined arteries
276 was not affected by epidural anesthesia.

277 ***3.2.4. Time-averaged maximum velocity of the AUT and AUM***

278 The TAMV of the ipsilateral AUT increased significantly after epidural
279 anesthesia from 137.79 ± 21.93 cm/s to 153.81 ± 25.73 cm/s and decreased after
280 xylazine from 141.18 ± 20.38 cm/s to 125.13 ± 18.80 cm/s (both $P < 0.001$). The
281 TAMV of the contralateral AUT decreased significantly after isoxsuprine and xylazine
282 from 118.44 ± 23.05 cm/s to 106.48 ± 21.38 cm/s and from 107.64 ± 23.05 cm/s to
283 91.58 ± 20.63 cm/s, respectively (both $P < 0.001$). The TAMV of the AUM was not
284 affected by any of the experimental drugs.

285 ***3.2.5. Blood flow volume in the AUT and AUM***

286 The BFV in the ipsilateral AUT increased after epidural anesthesia from
287 214.68 ± 39.60 cm^3/s to 232.06 ± 48.36 cm^3/s and Xylazine caused a decrease in
288 BFV from 216.60 ± 31.77 cm^3/s to 191.87 ± 35.63 cm^3/s (Fig. 5a; both $P < 0.01$). In
289 the contralateral AUT, isoxsuprine caused an increase from 90.20 ± 29.75 cm^3/s to
290 101.99 ± 42.25 cm^3/s) and xylazine resulted in a decrease from 84.66 ± 33.96 cm^3/s
291 to 79.37 ± 34.21 cm^3/s (Fig. 5b; both $P \leq 0.05$). The combined BFV in both AUT was
292 affected by all 3 drugs (Fig. 5c). Isoxsuprine caused an increase from 301.72 ± 73.41
293 cm^3/s to 317.44 ± 73.65 cm^3/s and epidural anesthesia caused an increase from
294 297.77 ± 41.70 cm^3/s to 315.53 ± 49.88 cm^3/s (both $P \leq 0.05$). Xylazine caused a
295 decrease in total BFV from 302.83 ± 37.66 cm^3/s to 271.81 ± 45.59 cm^3/s. ($P <$
296 0.001).

297 Isoxsuprine caused the BFV in the AUM to increase from 72.87 ± 19.76 cm^3/s
298 to 82.01 ± 24.22 cm^3/s (Fig. 5d; $P \leq 0.05$) but epidural anesthesia and xylazine had
299 no effect.

300

301 ## *3.3. Comparison of the experimental drugs*

302 ***3.3.1. Pulse rate in cows and foetuses***

303 The effects of the 3 drugs on PR of the cows differed. Isoxsuprine caused the
304 highest pulse rate (132.6 ± 10.3 min^{-1}) followed by epidural anesthesia (79.9 ± 6.4
305 min^{-1}) and xylazine (65.5 ± 5.6 min^{-1}) (Fig. 6a; all $P < 0.0001$).

306 Isoxsuprine caused a higher PR in the AUM (163.1 ± 23.6 min^{-1}) than epidural
307 anesthesia (113.7 ± 10.7 min^{-1}; Fig. 6b; $P < 0.01$) and xylazine (110.5 ± 9.8 min^{-1}; $P <$
308 0.001).

309 ***3.3.2. Diameter of the AUT and AUM***

310 The 3 drugs had no effect on DM of the ipsilateral AUT. The DM of the
311 contralateral AUT was greater after isoxsuprine (10.88 ± 1.99 mm) than after epidural
312 anesthesia (9.64 ± 1.99 mm, $P < 0.01$).

313 The DM of the AUM was greater after isoxsuprine (10.44 ± 1.00 mm) than
314 after epidural anesthesia (9.77 ± 0. 79 mm) and xylazine (9.81 ± 1.23 mm) (both $P <$
315 0.01).

316 ***3.3.3. Resistance index of the AUT and AUM***

317 The effects of the medication on RI of both the ipsi- and contralateral AUT
318 differed significantly (all P<0.001). The smallest RI occurred after isoxsuprine
319 (ipsilateral 0.398 ± 0.040, contralateral 0.498 ± 0.079) followed by epidural
320 anesthesia (ipsilateral 0.482 ± 0.055, contralateral 0.582 ± 0.082) and xylazine
321 (ipsilaterale 0.558 ± 0.074, contralateral 0.645 ± 0.080).
322 In the AUM, the RI was smaller after isoxsuprine (0.481 ± 0.080) than after
323 epidural anesthesia (0.594 ± 0.076) and xylazine (608 ± 0.062) (both P< 0.001).

324 ***3.3.4. Time-averaged maximum velocity in the AUT and AUM***

325 The effect of xylazine on TAMV in both AUT differed significantly from the
326 effects of epidural anesthesia and isoxsuprine (all P < 0.001). After xylazine, the
327 TAMV in the ipsilateral (125.13 ± 10.80 cm/s) and contralateral AUT (91.58 ± 20.63
328 cm/s) were smaller than after epidural anesthesia (ipsilateral 153.81 ± 25.73 cm/s,
329 contralateral 109.03 ± 23.99 cm/s) and after isoxsuprine (ipsilateral 145.99 ± 27.08
330 cm/s, contralateral 106.48 ± 21.38 cm/s).
331 The TAMV in the AUM after isoxsuprine (95.45 ± 22.94 cm/s) was greater than
332 after xylazine (81.829 ± 17.51 cm/s, P ≤ 0.05).

333 ***3.3.5. Blood flow volume in the AUT and AUM***

334 The BFV in the ipsilateral AUT was greater after epidural anesthesia (232.06 ±
335 48.36 cm^3/s) and after isoxsuprine (224.25 ± 48.44 cm^3/s) than after xylazine (191.87
336 ± 35.63 cm^3/s, Fig. 7a, both P < 0.001). The BFV in the contralateral AUT was
337 greater after isoxsuprine (101.98 ± 42.25 cm^3/s) than after epidural anesthesia (83.46
338 ± 35.02 cm^3/s, P ≤ 0.05) and xylazine (79.37 ± 34.21 cm^3/s, Fig. 7b, both P < 0.01).
339 The combined BFV in both AUT was greater after isoxsuprine (326.23 ± 90.68
340 cm^3/s) than after xylazine (271.24 ± 69.84 cm^3/s, Fig. 7c, P ≤ 0.05).
341 The BFV in the AUM was greater after isoxsuprine (82.01 ± 24.22 cm^3/s) than
342 after xylazine (68.68 ± 19.65 cm^3/s, P < 0.001) and epidural anesthesia (63.72 ±
343 20.00 cm^3/s, Fig. 7d; P ≤ 0.05).
344
345

346 # 4. Discussion

347 ## *4.1. Feasibility of measurements*

348 Because an intestinal spasmolytic was not used in the cows during transrectal
349 sonographic examinations, intestinal movements made it difficult to place the
350 transducer adjacent to the AUT for an extended length of time, particularly before the
351 experimental drugs were given. Five to 8 repeated measurements were therefore
352 made, between which the transducer was temporarily removed from the measuring
353 site, and then reapplied. This enabled us to overcome problems caused by
354 peristalsis, and blood flow could be evaluated without the use of artifact-causing
355 medication. The same technique was used after medication. Scanning of the AUM
356 was at times difficult or even impossible after xylazine and close to the due date
357 because of an increase in uterine contractions. Both of these factors caused the
358 umbilical blood vessels to be situated far from the abdominal wall and beyond the
359 depth of penetration of the transducer.
360

361 ### *4.2. Analysis of sonographic measurements*

362 In human medicine, Doppler sonographic examination of the uterine and
363 umbilical arteries is instrumental for monitoring fetal well-being [30,31]. The
364 hemodynamic parameters are interpreted in relation to the stage of gestation.
365 Monthly monitoring of the uterine arteries for diameter, TAMV, BFV, and RI was also
366 done in cows and revealed marked changes throughout gestation [6, 8]. There were
367 local adaption processes but also indications that the changes documented in the
368 uterine arteries are changes that affect the entire cardiovascular system. We
369 observed an increase in maternal pulse rate of 7.6% during the last 4 weeks of
370 gestation. An analogous increase also occurred in pregnant women and was
371 interpreted as a sequel to the increase in blood volume, which accounts for up to
372 45% throughout the entire gestation period. In agreement with other studies, we did
373 not observe an increase in fetal pulse rate, even though an increased demand on the
374 cardiovascular system during fetal development appears obvious [8,32]. This
375 demand is most likely met instead by the increasing size and efficiency of the heart in
376 bovine as well as human fetuses [33,34].
377 The diameters of the AUT and AUM did not change significantly during the
378 study period, which was in agreement with another study in which no diameter
379 changes were seen in cows from week 37 to 39 of gestation [8,35]. However, the
380 mean DM of the contralateral AUT was significantly greater in week -3 (3.2 %) and -2

(3.0 %) than in the last week of the study period. This may not have been biologically relevant because the TAMV, BFV and RI of this artery did not undergo corresponding changes during the same period. The mean RI of the ipsilateral AUT was 7% smaller in the week of calving than in the first week of the study. A continuous decrease of 35% in the RI of both AUT occurred in cattle during the first 28 weeks of gestation, but no further decrease was noted at monthly measurements during the remainder of gestation [6]. Likewise, there was no decrease in RI in cows from week 37 to week 39 of gestation [38]. The fall in RI represents an adaption to growing demands on gas exchange and transfer of nutrients and metabolites during the last trimester of gestation [36-38]. Structural changes in blood vessels of the human fetoplacental unit are associated with increased vascular elasticity and result in a reduction of vascular resistance [39]. The ability to increase vascular elasticity under the influence of the pressure within the vessels can also be affected by maternal factors such as diseases, age or parity. The mechanisms involved in the decrease in RI in pregnant cows remain unknown. Whether parity could have an influence on the compliance of the AUT in cows, which we could not analyze due to the large age differences between the cows, and changes in RI indicate a pregnancy disorder as they do in women needs further study.

4.3. Effects of medication on blood flow in the AUT and AUM

4.3.1. Isoxsuprine

After administration of isoxsuprine, the PR increased an average of 64% in the AUT and 37% in the AUM; in some cows, the PR even doubled. All cows had other adverse effects including droopy eyelids, anxiousness, insecure stance, shifting weight in all 4 legs and muscle tremors, mostly in the triceps muscle. These adverse effects are common after intravenous administration of isoxsuprine [40]. A positive chronotropic effect of isoxsuprine was thought to account for the increase in pulse rate in the cows and fetuses. In addition, isoxsuprine is a β-adrenergic agonist that causes vasodilatation in peripheral blood vessels by stimulating β2-receptors [41], which in turn can lead to a compensatory increase in heart rate.

In our study, the vasodilatory effect of isoxsuprine was limited to the contralateral AUT, in which the diameter increased by an average of 10%. It is not known why the diameter of the ipsilateral AUT did not increase in diameter during the study period and why it failed to dilate after administration of isoxsuprine. The

ipsilateral AUT was considerably larger than the contralateral AUT in the last week of gestation (14.03 ± 1.27 mm versus 9.77 ± 2.09 mm). It is possible that the capacity of the former to dilate in response to isoxsuprine was restricted because of the massive increase in diameter that had already occurred during gestation before the study started. Because the RI decreased significantly in the AUT and AUM, it can be concluded that isoxsuprine had a dilatory effect on the arterioles of the maternal as well as the fetal placenta. Compared with other arterial blood vessels, arterioles have the thickest smooth muscle layer relative to the luminal diameter and therefore have the greatest potential to respond to isoxsuprine [42]. After isoxsuprine, the RI decreased by 20.0% and 11.2% in the ipsilateral and contralateral AUT and by 21.3% in the AUM. A similar effect of β-sympathomimetic drugs on the RI of uterine and umbilical arteries occurred in pregnant women [43,44], in which these drugs are used for prevention of preterm birth and for intrauterine resuscitation [16]. The effect of the tocolytic drug ritodrine on the systolic-to-diastolic (S/D) ratio of the AUT and AUM was investigated in an early study of pregnant women [45]. Ritodrine is another β_2- adrenergic receptor agonist, and the S/D ratio describes the vascular resistance of the placental vascular bed distal to the point of measurement, similar to the RI. The S/D ratio of both the AUT and AUM decreased after ritodrine. A study of the effect of another β_2-mimetic drug, terbutaline, on fetal perfusion produced similar results [43].

A decrease of 10% in TAMV in the contralateral AUT was the only significant effect of isoxsuprine on blood flow velocity. This may have been related to the increase in diameter of the blood vessel because the continuity equation dictates that flow velocity decreases with increasing vessel diameter.

Beta-sympathomimetics are used in human medicine to control excessive uterine contractions or to bridge the time until a caesarean section can be started [16]. These so-called intrauterine resuscitative measures are instituted to reverse or prevent fetal hypoxia by ensuring adequate uterine perfusion. Judging by the increase in BFV in both the contralateral AUT and AUM after isoxsuprine, this effect seems to also have occurred in our study. Although this effect was not apparent in the ipsilateral AUT – presumably related to the failure to dilate – the combined BFV in both AUT increased by 5.2% and indicated an increase in uterine perfusion. The BFV in the AUM also increased by 12.5%, but it is not known whether this translated into an increased blood flow in the umbilical veins and thus to the fetus.

449 The distinct increase in fetal PR after isoxsuprine indicated increased
450 workload of the fetal heart and, in turn, increased myocardial oxygen requirement.
451 This could add to hypoxia in a fetus that is already compromised in later stages of
452 pregnancy or during birth.

453

454 ***4.3.2. Epidural anesthesia***

455 To our knowledge, the effect of epidural anesthesia on fetal well-being has not
456 been investigated in cattle. Epidural anesthesia is typically used in bovine obstetrics
457 for mutation procedures for the relief of dystocia, or during caesarean section to
458 control excessive uterine contractions. In this study, it resulted in a significant
459 decrease in pulse rate in the contralateral AUT and in a marked decrease in the
460 ipsilateral AUT. We explained this discrepancy by the fact that the two arteries were
461 not examined at exactly the same time. Although all cows were accustomed to
462 frequent transrectal examinations and tolerated the examinations well, the epidural
463 anesthesia could have alleviated stress and thus lowered the pulse rate.
464 Epidural anesthesia did not affect the RI in the present study, and its effect on
465 uterine and fetal perfusion in humans is controversial. In a study involving normal
466 women in labor, epidural anesthesia did not affect the S/D ratio [46], whereas a
467 significant decrease in peripheral vascular resistance in the AUM and uteroplacental
468 vasculature was noticed in another study and interpreted as improved perfusion [47].
469 Because epidural anesthesia may be associated with peripheral vasodilation
470 lowering blood pressure, a fluid preload is usually given in human medicine to
471 prevent hypovolemia [48].
472 Epidural anesthesia increased uterine BFV by 6% in the absence of significant
473 changes in the RI of the AUT and AUM. This can be explained by the 8% increase in
474 BFV in the ipsilateral AUT, which occurred despite a 1.4% decrease in diameter. The
475 BFV did not increase in the contralateral AUT. Conversely, epidural anesthesia in
476 ewes using nesacaine did not affect uterine blood flow [49]. Epidural anesthesia was
477 also used in Doppler sonographic studies to facilitate transrectal positioning of the
478 transducer and to increase accuracy of the results. However, our findings indicate
479 that measurements made with epidural anesthesia may be fraught with artifacts and
480 may differ from those obtained from untreated animals.

Epidural anesthesia may have potential usefulness for intrauterine resuscitation because it improves uterine perfusion without adversely affecting umbilical blood flow.

4.3.3. Xylazine

The use of the α-2 sympathomimetic drug xylazine in the last trimester is problematic in cows because of the risk of abortion [50,51]. Despite this limitation, sedation of cattle in late pregnancy or with dystocia is sometimes required. Xylazine has local uterine as well as systemic adverse effects and its use during dystocia or caesarean section may cause critical fetal hypoxia [52]. In agreement with another study [40], the PR in our cows fell by 17% and the fetal PR by 6%. In pregnant goats, the blood pressure in the femoral artery fell after the administration of xylazine [22]. The combined effects of decreased heart rate and lowered blood pressure may result in hypoxia in peripheral organs. Compensatory reduction in blood vessel diameter to counteract this effect did not occur in our cows; the diameter did not change in the ipsilateral AUT and even increased by 4% in the contralateral AUT. As a result, the TAMV decreased after xylazine by 11% in the ipsilateral AUT and by 15% in the contralateral AUT, causing a reduction in ipsilateral BFV by 11%, in contralateral BFV by 6.2%, and in combined BFV in both AUT by 10%.

The BFV of the internal iliac artery of pregnant heifers was not reduced after intramuscular administration of xylazine at the same dose as in the present study, but the BFV was lowered by 40% after 10 minutes [53]. It is possible that a more pronounced fall in BFV would have been detected in the present study, if measurements had been made sooner after intravenous xylazine administration. When xyalzine was given to pregnant cows intravenously at twice the dose we used, a maximum reduction in BFV of almost 60% was measured 5 minutes after medication, and the BFV remained below baseline 45 minutes later [52]. In the same study, blood flow and vascular resistance in the uterine artery changed in opposite directions; changes in vascular resistance reached a maximum of 156% 5 minutes after medication and remained increased 45 minutes later. Although it is a measure of vascular resistance distal to the point of examination, the RI increased by 13% and 10%, respectively, in the ipsilateral and contralateral AUT in our study. This was interpreted as a peripheral vasoconstrictive effect in the vascular bed of various organs, which is typical for α-2 adrenoreceptor agonists such as xylazine [54]. Thus,