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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 (AUT) and the fetal portion from the umbilical arteries (AUM). Placental perfusion is crucial for fetal development and undergoes adaptive changes during pregnancy according to the 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 4 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 AUT ipsilateral and contralateral to the pregnant horn and one AUM three times per week during the last 4 weeks of gestation. This was followed by the randomized administration of one of the three following experimental drugs per day: isoxsuprine (200 mg/cow, iv), xylazine (2 mg/100 kg, iv), 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 2 weeks of the study period. Uterine blood flow volume increased after isoxsuprine by 5% and after epidural anesthesia by 6% (both  $P = 0.05$ ) and decreased after xylazine by 10% ( $P < 0.001$ ). Isoxsuprine was the only drug that elevated the blood flow volume in the AUM ( $P = 0.05$ ). 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 reported that Doppler sonographic measurements of uterine and AUM change little in the last month of pregnancy in the cow. Isoxsuprine and epidural anesthesia with lidocaine have the potential to improve uterine perfusion.

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

4  
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10

11 **Abstract**

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23 drugs per day: isoxsuprine (200 mg/cow, intravenously), xylazine (2 mg/100 kg,  
24 intravenously) and lidocaine for epidural anesthesia (100 mg/cow). Doppler  
25 sonographic examination was repeated 30 minutes after medication.

26 Maternal pulse rate increased during the study period ( $P < 0.001$ ) and the  
27 diameter of the contralateral AUT was smaller in the last week before birth than in the  
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29 the last week than in the first two weeks of the study period. Uterine blood flow

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

39 *Keywords: Cow; fetus; gestation; ultrasound; Doppler ultrasound; intrauterine*  
40 *resuscitation*

41

42

## 43 **1. Introduction**

44

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

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

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

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

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

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

80 In human medicine, a variety of measures are used to determine and prevent  
81 fetal hypoxia. These include prepartal and peripartal monitoring of uterine, placental  
82 and fetal perfusion but also pharmacological interventions to improve uterine  
83 perfusion in pregnant women and women giving birth [16]. These steps are referred  
84 to as intrauterine resuscitation and may include the administration of tocolytic agents  
85 to control excessive uterine contractions, in cases of fetal growth restriction or  
86 chronic asphyxia [17]. To our knowledge, analogous studies investigating the positive  
87 and adverse effects of commonly used drugs on uterine perfusion in pregnant cows  
88 have not been published. The drugs most commonly used in bovine obstetrics are  
89 lidocaine for epidural anesthesia, and isoxsuprine and xylazine. Isoxsuprine, a  $\beta_1$ -  
90 and  $\beta_2$ -adrenomimetic drug, is used to relax the uterus to facilitate fetal mutations or  
91 to exteriorize the uterus during Caesarean section. Isoxsuprine relaxes the smooth  
92 musculature of the uterus and blood vessels and has a peripheral vasodilatory effect  
93 [18,19]. It stimulates  $\beta_2$ -receptors to activate adenylyl cyclase, which results in an  
94 increase in myometrial cAMP and thus a decrease in uterine contractility. Isoxsuprine  
95 also affects phosphodiesterase, which converts cAMP to AMP [20,21].

96 Xylazine has the opposite effect. A study in pregnant goats showed that it  
97 stimulates postsynaptic uterine  $\alpha_2$ -receptors causing an increase in uterine tone and  
98 a decrease in uterine arterial blood flow [22]. It is used for sedation of fractious  
99 animals and also has analgesic and muscle-relaxant properties [23].

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

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

116  
117

## 118 **2. Materials and Methods**

### 119 **2.1 *Animals***

120 Seven Braunvieh and two Red Holstein cows, which ranged in age from 3 to  
121 17 years and weighed between 720 and 866 kg, were used. Parity ranged from  
122 second to 14th. The cows were brought to our clinic 4 weeks before the calculated  
123 due date. They were kept in tie stalls, bedded with straw and fed hay, grass silage  
124 and water ad libitum. They had daily access to pasture. The cows were moved to a  
125 straw-bedded pen upon the first signs of calving and remained there until after  
126 delivery of the placenta.

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

## 131 **2.2 Study design**

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

141           One of the three experimental drugs was then administered. The other two  
142 drugs were given on the other two examination days of the same week; a given drug  
143 was used only once per cow per week. The order of drug administration varied  
144 among cows and changed weekly. The drugs were administered according to the  
145 recommendations of the manufacturer. Isoxsuprine (Isoxsuprini hydrochloridum  
146 10 mg/ml, Degraspasmin, Graeub, Bern, Switzerland) was administered into a jugular  
147 vein at a dose of 200 mg/cow. Xylazine (20 mg/ml, Xylazin Streuli, Streuli Pharma,  
148 Uznach, Switzerland) was given into a coccygeal vene at the tail site at a dose of  
149 0.02 mg/kg. Epidural anesthesia was achieved with 5 ml 2% lidocaine (Lidocain  
150 20 mg/ml, Lidocain 2% Chassot, Vétoquinol, Ittingen, Switzerland) injected into the  
151 sacrococcygeal space or between the first two coccygeal vertebrae. B mode and  
152 Doppler sonographic examination of both AUT and one AUM was carried out 30  
153 minutes after medication.

154

## 155 **2.3. B mode and Doppler sonography**

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

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

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

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

185

#### 186 **2.4. Data collection, analysis and statistics**

187 The images were visually assessed using Photoshop Album Starter Edition  
188 3.0 (Adobe, Zürich, Switzerland) and selected for analysis. Three sharp gray-scale  
189 images of arteries with the most circular cross-section were selected and the arterial  
190 diameter (DM) was measured and the mean calculated. Four spectral displays with  
191 regular representative waveforms were selected and analyzed using PixelFlux  
192 Scientific (Chameleon Software, Freiburg i. Br., Germany). The time-averaged

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

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

205 The program StatView 5.0 (SAS Institute, Wangen, Switzerland) was used for  
206 statistical calculations. For each variable, means and standard deviations were  
207 calculated before and after the administration of each drug. Changes in the  
208 measured variables during the study period were analyzed using ANOVA for  
209 repeated measures. Fisher's PLSD test was used to analyze differences among  
210 measurements obtained in different weeks. Differences between measurements  
211 before and after the administration were analyzed using a paired  $t$ -test. For  
212 comparison among drugs, differences in measurements obtained after the  
213 administration of different drugs were also analyzed using a paired  $t$ -test. A  $P$ -value  $\leq$   
214 0.05 was considered significant.

215

## 216 **2.5 Animal experiment permission**

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

219

220

## 221 **3. Results**

222



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

226

### 227 ***3.1. Changes in B mode and Doppler sonographic variables during*** 228 ***the last 4 weeks of gestation***

#### 229 ***3.1.1. Pulse rate in cows and fetuses***

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

#### 233 ***3.1.2. Diameter of the AUT and AUM***

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

#### 239 ***3.1.3. Resistance index, blood flow velocity and blood flow volume in the AUT*** 240 ***and AUM***

241 The changes in RI of the AUT and AUM over the study period were not  
242 significant. In the ipsilateral AUT, the RI ranged from  $0.472 \pm 0.059$  to  $0.513 \pm 0.042$ ,  
243 in the contralateral AUT from  $0.566 \pm 0.085$  to  $0.590 \pm 0.068$  and in the AUM from  
244  $0.595 \pm 0.087$  to  $0.620 \pm 0.052$ . The RI of the ipsilateral AUT was significantly greater  
245 in week -4 ( $0.513 \pm 0.042$ ) and -3 ( $0.508 \pm 0.052$ ) of the study period than in the last  
246 week ( $0.472 \pm 0.059$ ;  $P \leq 0.05$ ).

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

249

### 250 ***3.2. Comparison of B mode and Doppler sonographic*** 251 ***measurements before and after medication***

252

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

254 The maternal PR, assessed in both AUT, increased after isoxsuprine from  
255  $80.8 \pm 6.5 \text{ min}^{-1}$  to  $132.0 \pm 10.5 \text{ min}^{-1}$  and decreased after xylazine from  $79.8 \pm 6.8$   
256  $\text{min}^{-1}$  to  $66.0 \pm 5.9 \text{ min}^{-1}$  (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  $81.4 \pm 6.7 \text{ min}^{-1}$  to  $79.7 \pm 6.4 \text{ min}^{-1}$ ; Fig. 2b;  $P < 0.01$ ). The pulse rate  
260 in the AUM increased after isoxsuprine from  $119.1 \pm 14.5 \text{ min}^{-1}$  to  $163.1 \pm 23.6 \text{ min}^{-1}$   
261 and decreased after xylazine from  $117.0 \pm 13.5 \text{ min}^{-1}$  to  $110.5 \pm 9.8 \text{ min}^{-1}$  (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  $14.1 \pm 1.1 \text{ mm}$  to  $13.9 \pm 1.3 \text{ mm}$  (Fig. 3a;  $P < 0.001$ ). The  
266 DM of the contralateral AUT increased after isoxsuprine from  $9.9 \pm 1.8 \text{ mm}$  to  $10.9 \pm$   
267  $2.0 \text{ mm}$  (Fig. 3b;  $P < 0.001$ ) and also after xylazine from  $9.8 \pm 2.1 \text{ mm}$  to  $10.2 \pm 2.0$   
268  $\text{mm}$  (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  $0.484 \pm 0.050$  to  $0.387 \pm 0.040$  and from  $0.561 \pm 0.058$  to  $0.498 \pm 0.079$ ,  
272 respectively (both  $P < 0.001$ ) and increased after xylazine from  $0.496 \pm 0.057$  to  
273  $0.558 \pm 0.074$  and from  $0.585 \pm 0.073$  to  $0.645 \pm 0.080$ , respectively (Figs. 4a, b;  
274 both  $P < 0.001$ ). The RI of the AUM decreased significantly after isoxsuprine from  
275  $0.611 \pm 0.077$  to  $0.481 \pm 0.080$  (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  $137.79 \pm 21.93 \text{ cm/s}$  to  $153.81 \pm 25.73 \text{ cm/s}$  and decreased after  
280 xylazine from  $141.18 \pm 20.38 \text{ cm/s}$  to  $125.13 \pm 18.80 \text{ cm/s}$  (both  $P < 0.001$ ). The  
281 TAMV of the contralateral AUT decreased significantly after isoxsuprine and xylazine  
282 from  $118.44 \pm 23.05 \text{ cm/s}$  to  $106.48 \pm 21.38 \text{ cm/s}$  and from  $107.64 \pm 23.05 \text{ cm/s}$  to  
283  $91.58 \pm 20.63 \text{ cm/s}$ , 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  $214.68 \pm 39.60 \text{ cm}^3/\text{s}$  to  $232.06 \pm 48.36 \text{ cm}^3/\text{s}$  and Xylazine caused a decrease in  
 288 BFV from  $216.60 \pm 31.77 \text{ cm}^3/\text{s}$  to  $191.87 \pm 35.63 \text{ cm}^3/\text{s}$  (Fig. 5a; both  $P < 0.01$ ). In  
 289 the contralateral AUT, isoxsuprine caused an increase from  $90.20 \pm 29.75 \text{ cm}^3/\text{s}$  to  
 290  $101.99 \pm 42.25 \text{ cm}^3/\text{s}$ ) and xylazine resulted in a decrease from  $84.66 \pm 33.96 \text{ cm}^3/\text{s}$   
 291 to  $79.37 \pm 34.21 \text{ cm}^3/\text{s}$  (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  $301.72 \pm 73.41$   
 293  $\text{cm}^3/\text{s}$  to  $317.44 \pm 73.65 \text{ cm}^3/\text{s}$  and epidural anesthesia caused an increase from  
 294  $297.77 \pm 41.70 \text{ cm}^3/\text{s}$  to  $315.53 \pm 49.88 \text{ cm}^3/\text{s}$  (both  $P \leq 0.05$ ). Xylazine caused a  
 295 decrease in total BFV from  $302.83 \pm 37.66 \text{ cm}^3/\text{s}$  to  $271.81 \pm 45.59 \text{ cm}^3/\text{s}$ . ( $P <$   
 296  $0.001$ ).

297 Isoxsuprine caused the BFV in the AUM to increase from  $72.87 \pm 19.76 \text{ cm}^3/\text{s}$   
 298 to  $82.01 \pm 24.22 \text{ cm}^3/\text{s}$  (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 ( $132.6 \pm 10.3 \text{ min}^{-1}$ ) followed by epidural anesthesia ( $79.9 \pm 6.4$   
 305  $\text{min}^{-1}$ ) and xylazine ( $65.5 \pm 5.6 \text{ min}^{-1}$ ) (Fig. 6a; all  $P < 0.0001$ ).

306 Isoxsuprine caused a higher PR in the AUM ( $163.1 \pm 23.6 \text{ min}^{-1}$ ) than epidural  
 307 anesthesia ( $113.7 \pm 10.7 \text{ min}^{-1}$ ; Fig. 6b;  $P < 0.01$ ) and xylazine ( $110.5 \pm 9.8 \text{ min}^{-1}$ ;  $P <$   
 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 ( $10.88 \pm 1.99 \text{ mm}$ ) than after epidural  
 312 anesthesia ( $9.64 \pm 1.99 \text{ mm}$ ,  $P < 0.01$ ).

313 The DM of the AUM was greater after isoxsuprine ( $10.44 \pm 1.00 \text{ mm}$ ) than  
 314 after epidural anesthesia ( $9.77 \pm 0.79 \text{ mm}$ ) and xylazine ( $9.81 \pm 1.23 \text{ mm}$ ) (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  $0.398 \pm 0.040$ , contralateral  $0.498 \pm 0.079$ ) followed by epidural  
320 anesthesia (ipsilateral  $0.482 \pm 0.055$ , contralateral  $0.582 \pm 0.082$ ) and xylazine  
321 (ipsilaterale  $0.558 \pm 0.074$ , contralateral  $0.645 \pm 0.080$ ).

322 In the AUM, the RI was smaller after isoxsuprine ( $0.481 \pm 0.080$ ) than after  
323 epidural anesthesia ( $0.594 \pm 0.076$ ) and xylazine ( $0.608 \pm 0.062$ ) (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 ( $125.13 \pm 10.80$  cm/s) and contralateral AUT ( $91.58 \pm 20.63$   
328 cm/s) were smaller than after epidural anesthesia (ipsilateral  $153.81 \pm 25.73$  cm/s,  
329 contralateral  $109.03 \pm 23.99$  cm/s) and after isoxsuprine (ipsilateral  $145.99 \pm 27.08$   
330 cm/s, contralateral  $106.48 \pm 21.38$  cm/s).

331 The TAMV in the AUM after isoxsuprine ( $95.45 \pm 22.94$  cm/s) was greater than  
332 after xylazine ( $81.829 \pm 17.51$  cm/s,  $P \leq 0.05$ ).

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

334 The BFV in the ipsilateral AUT was greater after epidural anesthesia ( $232.06 \pm$   
335  $48.36$  cm<sup>3</sup>/s) and after isoxsuprine ( $224.25 \pm 48.44$  cm<sup>3</sup>/s) than after xylazine ( $191.87$   
336  $\pm 35.63$  cm<sup>3</sup>/s, Fig. 7a, both  $P < 0.001$ ). The BFV in the contralateral AUT was  
337 greater after isoxsuprine ( $101.98 \pm 42.25$  cm<sup>3</sup>/s) than after epidural anesthesia ( $83.46$   
338  $\pm 35.02$  cm<sup>3</sup>/s,  $P \leq 0.05$ ) and xylazine ( $79.37 \pm 34.21$  cm<sup>3</sup>/s, Fig. 7b, both  $P < 0.01$ ).

339 The combined BFV in both AUT was greater after isoxsuprine ( $326.23 \pm 90.68$   
340 cm<sup>3</sup>/s) than after xylazine ( $271.24 \pm 69.84$  cm<sup>3</sup>/s, Fig. 7c,  $P \leq 0.05$ ).

341 The BFV in the AUM was greater after isoxsuprine ( $82.01 \pm 24.22$  cm<sup>3</sup>/s) than  
342 after xylazine ( $68.68 \pm 19.65$  cm<sup>3</sup>/s,  $P < 0.001$ ) and epidural anesthesia ( $63.72 \pm$   
343  $20.00$  cm<sup>3</sup>/s, Fig. 7d;  $P \leq 0.05$ ).

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

381 (3.0 %) than in the last week of the study period. This may not have been biologically  
382 relevant because the TAMV, BFV and RI of this artery did not undergo corresponding  
383 changes during the same period. The mean RI of the ipsilateral AUT was 7% smaller  
384 in the week of calving than in the first week of the study. A continuous decrease of  
385 35% in the RI of both AUT occurred in cattle during the first 28 weeks of gestation,  
386 but no further decrease was noted at monthly measurements during the remainder of  
387 gestation [6]. Likewise, there was no decrease in RI in cows from week 37 to week  
388 39 of gestation [38]. The fall in RI represents an adaption to growing demands on gas  
389 exchange and transfer of nutrients and metabolites during the last trimester of  
390 gestation [36-38]. Structural changes in blood vessels of the human fetoplacental unit  
391 are associated with increased vascular elasticity and result in a reduction of vascular  
392 resistance [39]. The ability to increase vascular elasticity under the influence of the  
393 pressure within the vessels can also be affected by maternal factors such as  
394 diseases, age or parity. The mechanisms involved in the decrease in RI in pregnant  
395 cows remain unknown. Whether parity could have an influence on the compliance of  
396 the AUT in cows, which we could not analyze due to the large age differences  
397 between the cows, and changes in RI indicate a pregnancy disorder as they do in  
398 women needs further study.

399

### 400 ***4.3. Effects of medication on blood flow in the AUT and AUM***

#### 401 ***4.3.1. Isoxsuprine***

402 After administration of isoxsuprine, the PR increased an average of 64% in the  
403 AUT and 37% in the AUM; in some cows, the PR even doubled. All cows had other  
404 adverse effects including droopy eyelids, anxiousness, insecure stance, shifting  
405 weight in all 4 legs and muscle tremors, mostly in the triceps muscle. These adverse  
406 effects are common after intravenous administration of isoxsuprine [40]. A positive  
407 chronotropic effect of isoxsuprine was thought to account for the increase in pulse  
408 rate in the cows and fetuses. In addition, isoxsuprine is a  $\beta$ -adrenergic agonist that  
409 causes vasodilatation in peripheral blood vessels by stimulating  $\beta$ 2-receptors [41],  
410 which in turn can lead to a compensatory increase in heart rate.

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

415 ipsilateral AUT was considerably larger than the contralateral AUT in the last week of  
416 gestation ( $14.03 \pm 1.27$  mm versus  $9.77 \pm 2.09$  mm). It is possible that the capacity of  
417 the former to dilate in response to isoxsuprine was restricted because of the massive  
418 increase in diameter that had already occurred during gestation before the study  
419 started. Because the RI decreased significantly in the AUT and AUM, it can be  
420 concluded that isoxsuprine had a dilatory effect on the arterioles of the maternal as  
421 well as the fetal placenta. Compared with other arterial blood vessels, arterioles have  
422 the thickest smooth muscle layer relative to the luminal diameter and therefore have  
423 the greatest potential to respond to isoxsuprine [42]. After isoxsuprine, the RI  
424 decreased by 20.0% and 11.2% in the ipsilateral and contralateral AUT and by  
425 21.3% in the AUM. A similar effect of  $\beta$ -sympathomimetic drugs on the RI of uterine  
426 and umbilical arteries occurred in pregnant women [43,44], in which these drugs are  
427 used for prevention of preterm birth and for intrauterine resuscitation [16]. The effect  
428 of the tocolytic drug ritodrine on the systolic-to-diastolic (S/D) ratio of the AUT and  
429 AUM was investigated in an early study of pregnant women [45]. Ritodrine is another  
430  $\beta_2$ -adrenergic receptor agonist, and the S/D ratio describes the vascular resistance  
431 of the placental vascular bed distal to the point of measurement, similar to the RI.  
432 The S/D ratio of both the AUT and AUM decreased after ritodrine. A study of the  
433 effect of another  $\beta_2$ -mimetic drug, terbutaline, on fetal perfusion produced similar  
434 results [43].

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

439 Beta-sympathomimetics are used in human medicine to control excessive  
440 uterine contractions or to bridge the time until a caesarean section can be started  
441 [16]. These so-called intrauterine resuscitative measures are instituted to reverse or  
442 prevent fetal hypoxia by ensuring adequate uterine perfusion. Judging by the  
443 increase in BFV in both the contralateral AUT and AUM after isoxsuprine, this effect  
444 seems to also have occurred in our study. Although this effect was not apparent in  
445 the ipsilateral AUT – presumably related to the failure to dilate – the combined BFV in  
446 both AUT increased by 5.2% and indicated an increase in uterine perfusion. The BFV  
447 in the AUM also increased by 12.5%, but it is not known whether this translated into  
448 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.



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

#### 484 **4.3.3. Xylazine**

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

499 The BFV of the internal iliac artery of pregnant heifers was not reduced after  
500 intramuscular administration of xylazine at the same dose as in the present study, but  
501 the BFV was lowered by 40% after 10 minutes [53]. It is possible that a more  
502 pronounced fall in BFV would have been detected in the present study, if  
503 measurements had been made sooner after intravenous xylazine administration.  
504 When xylazine was given to pregnant cows intravenously at twice the dose we used,  
505 a maximum reduction in BFV of almost 60% was measured 5 minutes after  
506 medication, and the BFV remained below baseline 45 minutes later [52]. In the same  
507 study, blood flow and vascular resistance in the uterine artery changed in opposite  
508 directions; changes in vascular resistance reached a maximum of 156% 5 minutes  
509 after medication and remained increased 45 minutes later. Although it is a measure  
510 of vascular resistance distal to the point of examination, the RI increased by 13% and  
511 10%, respectively, in the ipsilateral and contralateral AUT in our study. This was  
512 interpreted as a peripheral vasoconstrictive effect in the vascular bed of various  
513 organs, which is typical for  $\alpha$ -2 adrenoreceptor agonists such as xylazine [54]. Thus,