

Research article

Doppler Sonography is A Useful Method to Assess the Effects of Maternal Anxiety on Intrauterine Fetal Growth in Pregnant Sheep and Goats

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ABSTRACT

Objective: The objective of this prospective study was to investigate whether Doppler- and B- mode sonography could be used to assess a possible influence of maternal temperament on fetal and pregnancy-related end points in small ruminants.

Method: On the basis of behavioural testing related to an anxiety-eliciting stimulus (arena test), 10 sheep and 9 goats were divided into two groups, namely more reactive/anxious (MR: 6 sheep/7 pregnancies; 5 goats/7 pregnancies) and less reactive/anxious (LR: 4 sheep/6 pregnancies; 4 goats/6 pregnancies). After mating, several end points were assessed by transrectal and/or transabdominal ultrasonography every 2 weeks (wk) from breeding to wk 20 of pregnancy. The resistance index (RI) as well as the pulsatility index (PI) of the maternal uterine (UtA) and the fetal umbilical arteries (UMA) were measured with non-invasive Doppler sonography. Additionally the fetal heart rate (FHR) was determined. Further parameters were measured with B-mode ultrasonography: diameter of amniotic vesicles (AVD; only wk 2-8), diameters of placentomes (PLD), umbilicus (UMD), fetal chest (FCHD) and orbita (FOD), as well as bi-orbital breadth (FBO), fetal occipito-snout length (FOSL), and metacarpal length (MCL).

Results: In sheep, UtA-PI was significantly higher in MR compared to LR ewes at pregnancy wk 6 ($P>0.01$), 10 ($P>0.05$) and 12 ($P>0.05$), while a tendency was recognized at wk 14 ($P=0.054$). The same was true for UtA-RI during the first 8 wk of pregnancy ($P<0.03$) when MR and LR animals were compared. Similarly, UMA-RI was higher in fetuses of MR than LR ewes at wk 14 ($P< 0.0003$) and 20 ($P< 0.02$) of pregnancy. The differences in UMA-PI reached significance at wk 6, 8, 10 and 20 ($P< 0.05$–0.003). Furthermore, AVD ($P< 0.03$, wk 2-8), FCHD ($P<0.002$, wk 8-18) as well as the UMD ($P<0.054$, wk 8-18) were higher in LR than MR pregnant ewes. In goats, UtA-RI and -PI were significantly influenced by reactivity/anxiety 58 of the dam (increase in MR, resp.; RI: wk 6, 16, 18 and 20, ($P<0.05 – 0.0001$; PI: = wk 4, 8, 18 59 and 20, $P<0.05$-0.01). Both UMA-RI and UMA-PI (wk 8-20, $P<0.01$ and<0.02, resp.) were higher in MR than LR animals. B-mode end points were similar to those in sheep: AVD ($P<0.01$, until wk 8), FCHD, PLD ($P<0.05$-0.005), and FMCL ($P<0.01$) were larger in LR than in MR goats during wk 8-18. Other fetometric parameters were not affected by temperament of the pregnant dam.

Conclusion: Using Doppler- and B-mode sonography, effects of maternal temperament on fetal growth, as well as maternal and umbilical blood flow in sheep and goats could be successfully demonstrated.

Keywords: Non-invasive colour; Doppler sonography; Anxiety; Uterus; Umbilical blood flow

Introduction

Many factors affect reproductive functions in small ruminants, including season, nutrition, social structure, and stress.[1-7] Additionally, maternal temperament seems to influence fecundity in small ruminants, since calm ewes produced more progeny due to higher ovulation rate,[7] and less lamb mortality was recognized in the first 3 days post-partum.[8] Effects of temperament on embryo and fetal development in small ruminants have not been investigated, although it is known that stress affects intrauterine life. It has been demonstrated that stress in the post-conceptional period (D2 and 3 after conception) induced shorter gestation periods after embryo transfer and affected fetal growth, although lamb birth weight was not significantly altered.[9]

In recent studies in humans, low offspring birth weight was attributed to exposure to stress during pregnancy, which may contribute to intrauterine fetal growth restriction.[10-13] Ultrasonography was used in women to characterize effects of maternal anxiety on fetal behavior and feto-maternal blood flow during pregnancy.[14-16] Based on Doppler sonography, it was emphasized that anxious women showed more abnormal variables of uterine artery blood flow than less anxious ones.[17] In that regard uterine artery PI and uteroplacental flow resistance were higher in stressed than in non-stressed pregnant women.[17-19] Additionally, it was recognized that the PI of fetal umbilical and cerebral vessels was increased in stressed versus less stressed women.[20] In contrast, Vythilingum et al. did not find significant correlations between measures of distress and anxiety and the umbilical artery PI or middle cerebral artery PI at any time-point.[18]

Pregnancy duration, birth weight, as well as fetal head circumference were all decreased by prenatal maternal stress and in females with more daily stressors and a depressed mood during the first trimester.[21] Similarly, femur length and circumference of the abdomen and head were reduced in babies with stressed mothers.[22,23] These effects were associated with increased fetal heart rate and adverse effects on fetometric parameters.[13]

The uterine artery resistance index (RI) increased as a result of maternal anxiety in pregnancy and was associated with delayed fetal development as well as a low birth weight.[17] Alternatively, it has been discussed that elevated prenatal cortisol levels induce increased glucose, free fatty acids and amino acid levels, resulting in enhanced early fetal growth of sheep, which might have been triggered via altered imprinted gene expression, activation of stress response pathways or disturbed embryo-maternal signalling.[9,24] Altered gene expression due to prenatal cortisol exposure, might also be accountable for fetal organ maturation in sheep.[25]

To best knowledge, the effects of maternal temperament on maternal, fetal and pregnancy related ultrasonic parameters have not been reported in small ruminants. The objective of the present prospective study was to investigate whether Doppler velocimetry variables are affected by maternal temperament which might result in altered fetal growth.

Material and Methods

Animals

These studies were conducted at the Institute of Reproductive Biology, Hannover, during two physiologic breeding seasons (2009/2010-2010/2011). Ten sheep and 9 goats were divided into two groups, namely more reactive/anxious (MR: 6 sheep/7 pregnancies; 5 goats/7 pregnancies) and less reactive/anxious (LR: 4 sheep/6 pregnancies; 4 goats/6 pregnancies) on the basis of Arena tests.[26,27]

The animals were kept on pasture, except during late autumn and winter, when they were housed in a barn with access to outside runs. During the latter interval, the base diet consisted of hay, mineral supplementation and water *ad libitum*, in addition to pelleted feed (amount adapted to the stage of pregnancy).

Experimental design

Animals were mated with a proven fertile male during behavioural estrus in October. Pregnancies were verified on 18 ± 2.8 in sheep and D14 ± 3.5 in goats by B-mode ultrasonography (Hitachi EUB 405, Hitachi Medical System, Japan). In sheep 6 singleton, 6 twin, 1 triplet pregnancies, and in goats 3 singleton, 8 twin and 2 triplet pregnancies were confirmed on D40-60 d after breeding. Fetal loss was not detected in any animal.

Doppler sonography

Measurements were conducted with a LOGIQ 5 Pro ultrasound device (General Electrics Healthcare, Solingen, Germany) equipped with a linear-array, multifrequency (5-10 MHz) transducer. The following variables were determined in uterine and umbilical arteries (Figures 1 and 2): resistance index (RI), and pulsatility index (PI). Additionally the fetal heart rate (FHR) was investigated. Doppler parameters were automatically measured. All parameters and techniques are described by Elmetwally.[28]

Investigations were performed in biweekly intervals at the same time of the day from wk 2 after breeding until parturition in both sheep and goats. Doppler imaging of all end points was completed within 20-30 min.

B-Mode sonography

From wk 2 after breeding until parturition, when possible, the following fetometric end points were measured with B-mode ultrasonography (Hitachi EUB 405, Hitachi Medical System, Japan), using a 5-7.5 MHz linear-array transducer: diameter of amniotic vesicles (AVD, according to Buckrell; wk 2-8), fetal chest (FCHD, according to Vahtiala et al.) and umbilical cord diameter close to the fetal body (UMD, according to Lee et al.) (Figure 2), metacarpal length (MCL, according to Greenwood and Bell), diameters of placentomes (PLD, according to Greenwood et al.), and orbita (FOD, according to Erdogan), as well as biorbital breadth (FBO, according to Lega et al.) and fetal occipito-snout length (FOSL, according to Kelly and

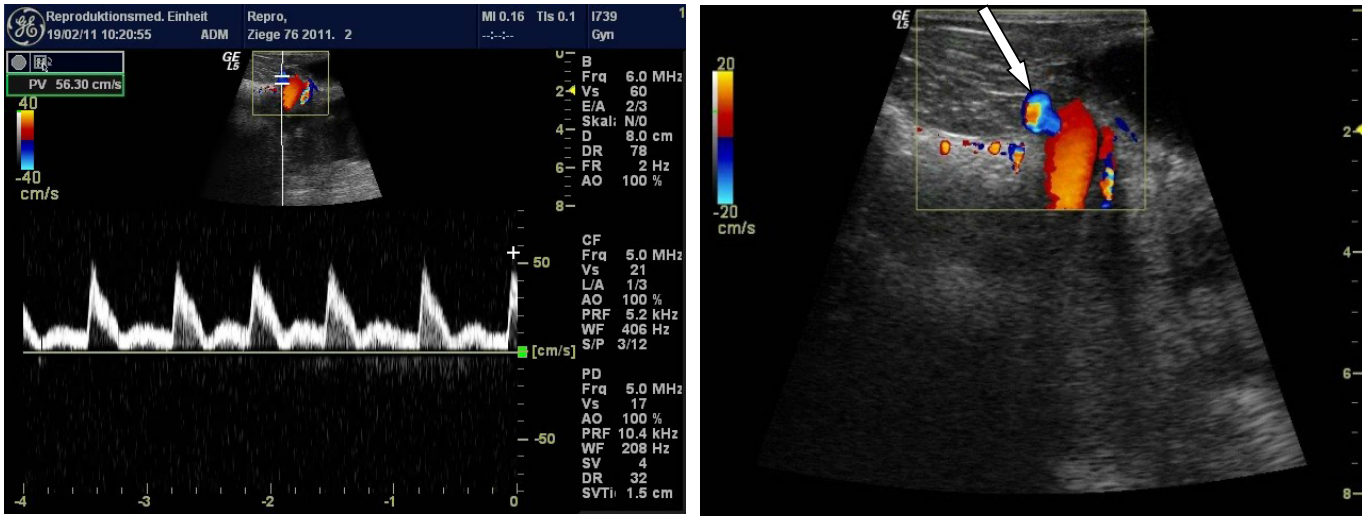


Figure 1A: Doppler measurements taken from the *A. umbilicalis* (goat, week 18 of pregnancy).
B: Arteria uterina goat (arrow), week 18 of pregnancy.

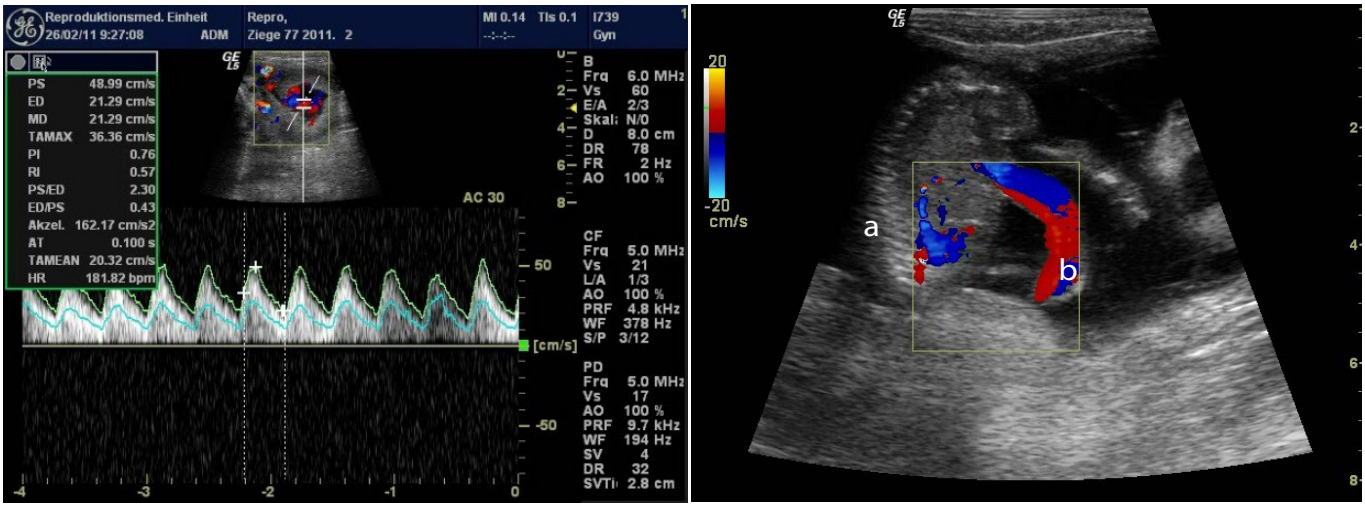
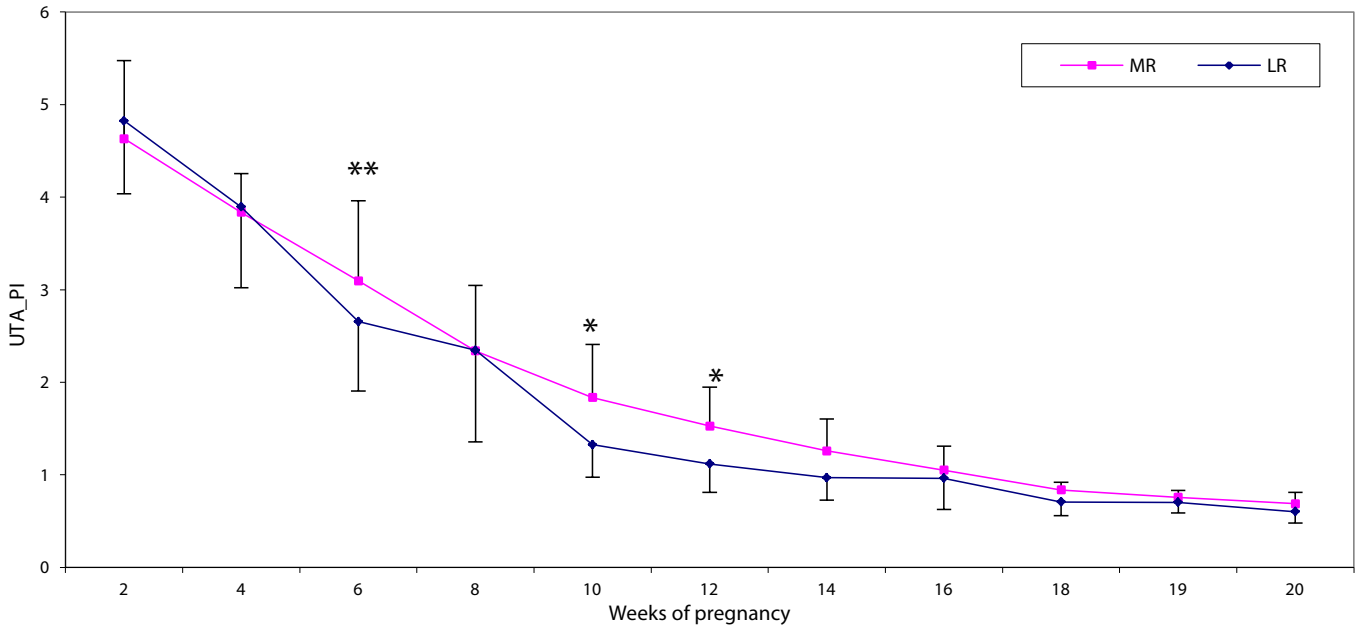


Figure 2A: Doppler measurements taken from *A. umbilicalis* (sheep, week 18 of pregnancy).
B: Chest (a) and umbilical cord with vessels (b) of an ovine fetus (week 10 of pregnancy).



Mean ± SD of UTA-PI during pregnancy in MR (n=7 pregnancies) and LR (n=6501 pregnancies) ewes. Values with asterisks differ between MR and LR sheep at the same 502 pregnancy week (* P<0.05 and ** P<0.01)

Figure 3: Effect of reactivity/anxiety on color Doppler parameters sheep.

Newnham).[29-35] These examinations required 20-30 min for each animal.

Statistical analysis

Statistical analyses were conducted separately for both sheep and goats. Goodness of fit for normal distribution of quantitative parameter was assessed by an analysis of the model-residuals using Q-Q-plots and Kolmogorov-Smirnov test.

Influence of maternal temperament on uterine and umbilical arteries blood flow as well as the fetal biometrical changes during pregnancy was analysed by two-way analysis of variance with behaviour-group (1=more reactive "MR"; 2=less reactive "LR") and week of gestation (2, 4,…, 18, 20) as main effects and interactions between both. Post hoc multiple pairwise comparisons were calculated with alpha-adjustment by Tukey to keep the family wise error rate. Statistical analyses were conducted with commercial statistical software (SAS®, version 9.2, SAS Institute, Cary, NC). For all analyses, $P \leq 0.05$ was defined as significant.

Results

Effect of maternal reactivity/anxiety on uterine and fetal umbilical blood flow and fetometric parameters

Doppler measurements in sheep: Measurements of the uterine and umbilical artery were successful in every attempt. In the case of twins or triplets, mean ± SD of the measurements was taken for analyses wherever necessary.

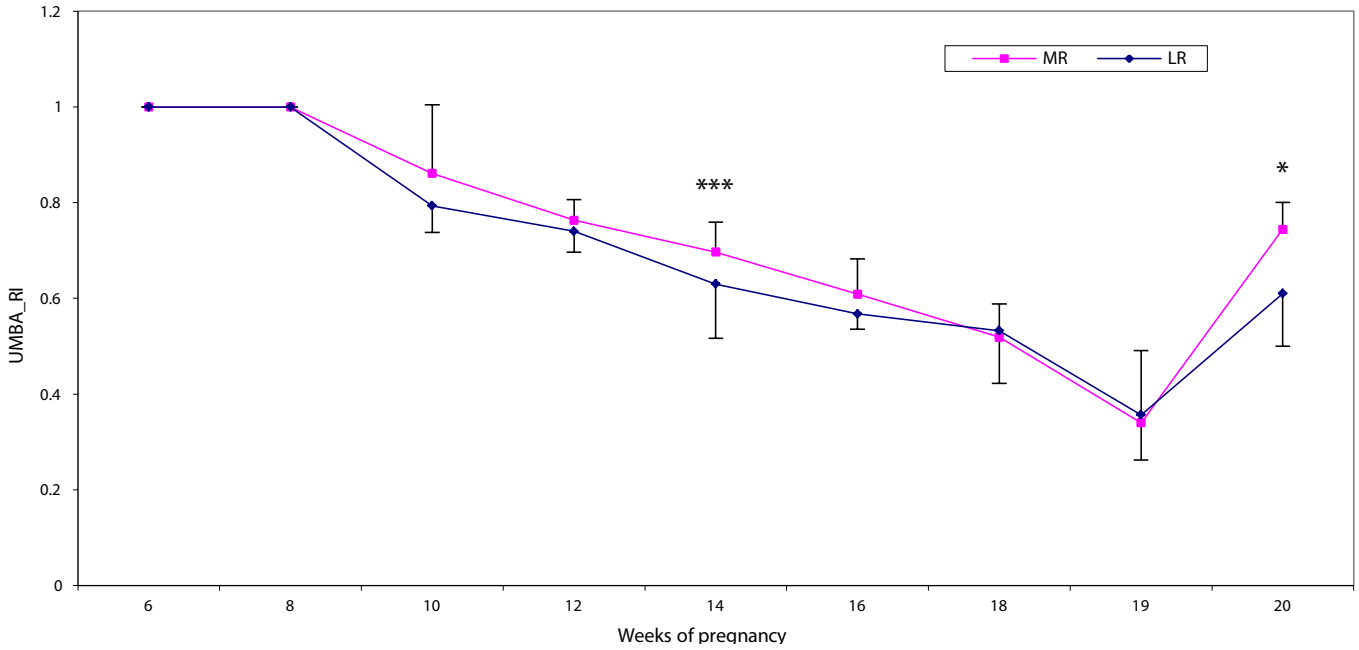
Uterine artery: UtA-PI (Figure 3) was higher during pregnancy in MR compared to LR ewes. The increase in UtA-PI was significant at pregnancy wk 6 (P>0.01), 10 (P>0.05) and 12 (P>0.05). Moreover, UtA-PI was tendentially higher at wk 14 (P=0.054). Furthermore, a higher UtA-RI was observed in MR ewes during the first 8 wk of pregnancy (P<0.03).

Umbilical artery: UMA-RI was higher in MR versus LR animals, especially at wk 14 (P<0.0003) and 20 (P<0.02) of pregnancy (Figure 4), while at the same time a tendentially higher UMA-PI (P=0.05, Figure 5) was observed. There was a significant increase of UMA-PI at wk 6, 8, 10 and 20 (P<0.05–0.003).

Fetal heart rate was not significantly (P=0.46) different between LR and MR ewes.

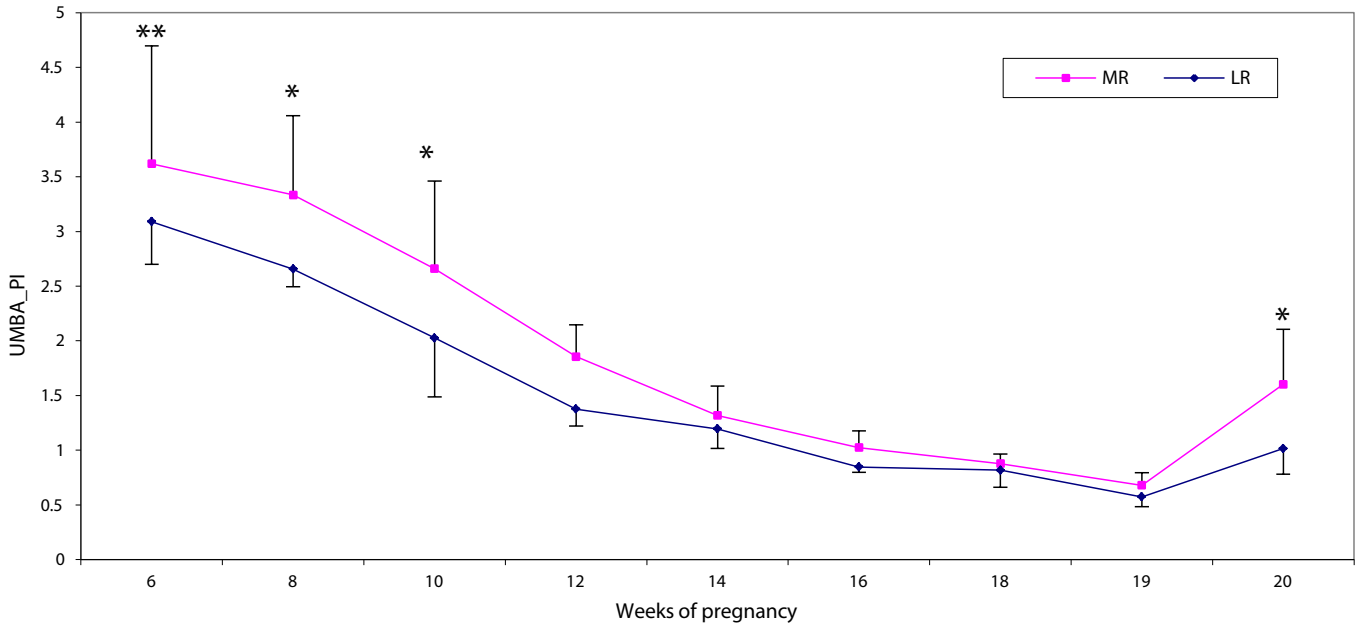
B-Mode measurements in sheep: B-mode measurements were successful in every attempt in the case of singleton and twins. In the case of twins or triplets, at least the measurements of two fetuses were taken (mean ± SD) for analyses. During the first 8 wk of pregnancy the amniotic vesicles diameter was larger in LR than in MR animals (P<0.03). Also, the fetal chest diameter (P<0.002) as well as the fetal umbilical diameter (P=0.05) was significantly larger in LR than MR pregnant ewes from wk 8-18. Other fetometric parameters (OBD, OS) did not significantly differ between MR and LR pregnant ewes (P>0.05). The fetal biometrical changes during pregnancy in all ewes, a linear increase (P<0.0001) in fetal parameters, including biparietal diameter, orbital diameter, chest diameter, umbilical cord diameter, fetal metacarpal length and placentome diameter occurred between wk 6 and wk 18 of gestation.

Doppler measurements in goats: Measurements of the uterine and umbilical artery were successful in every attempt.



Values with asterisks differ between MR and LR sheep at the same pregnancy week (*** P<0.0003 and * P<0.02)

Figure 4: Mean ± SD of UMA-RI during pregnancy in MR (n=7 pregnancies) and LR (n=6 pregnancies) ewes.



Values with asterisks differ between MR and LR sheep at the same 515 pregnancy week (* P<0.05 and ** P<0.01)

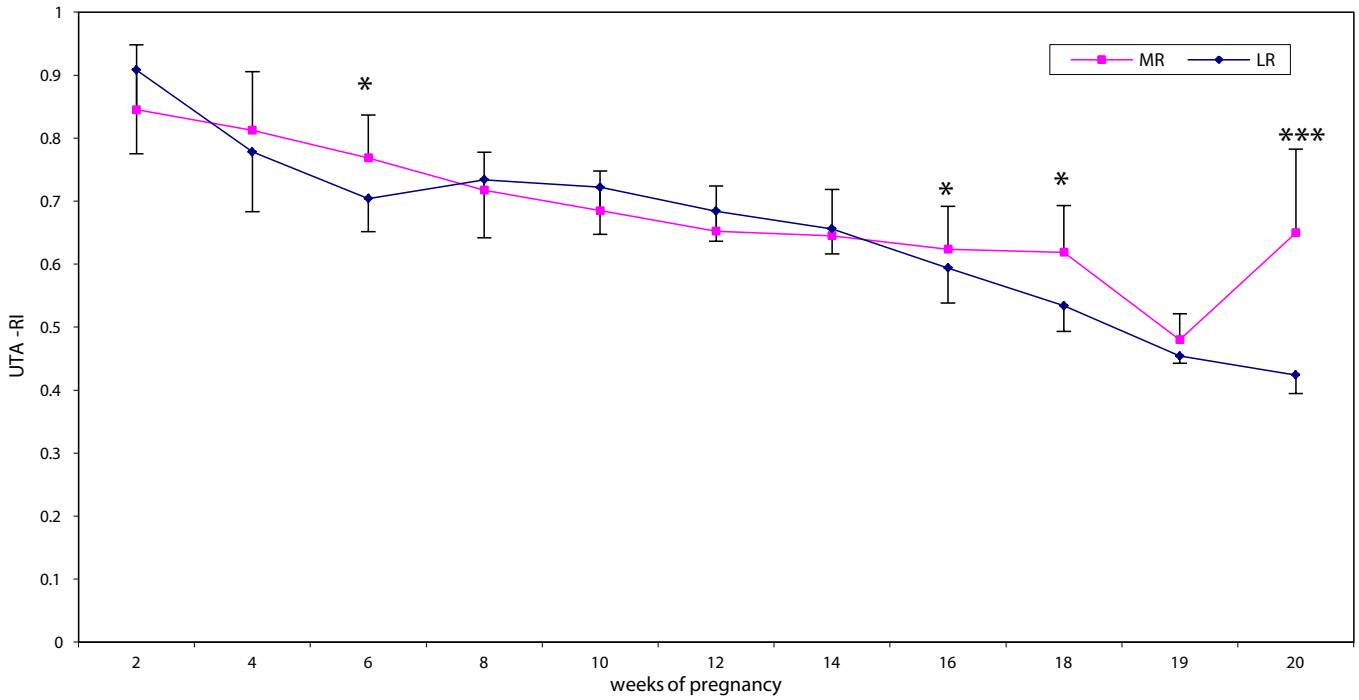
Figure 5: Mean ± SD of UMA-PI during pregnancy in MR (n=7 pregnancies) and LR (n=6514 pregnancies) ewes.

In the case of twins or triplets, mean ± SD of the measurements was taken for analyses wherever necessary.

Uterine artery: Significant differences related to UtA-RI were seen between MR and LR does especially at wk 6, 16, 18 and 20 (P<0.05–0.0001, Figure 6) of pregnancy, while in UtA-PI a tendency (0.05<P<0.10) was recognized (P=0.058; Figure 7). Significant differences were seen at wk 4, 8, 18 and 20 of pregnancy (P<0.05–0.01).

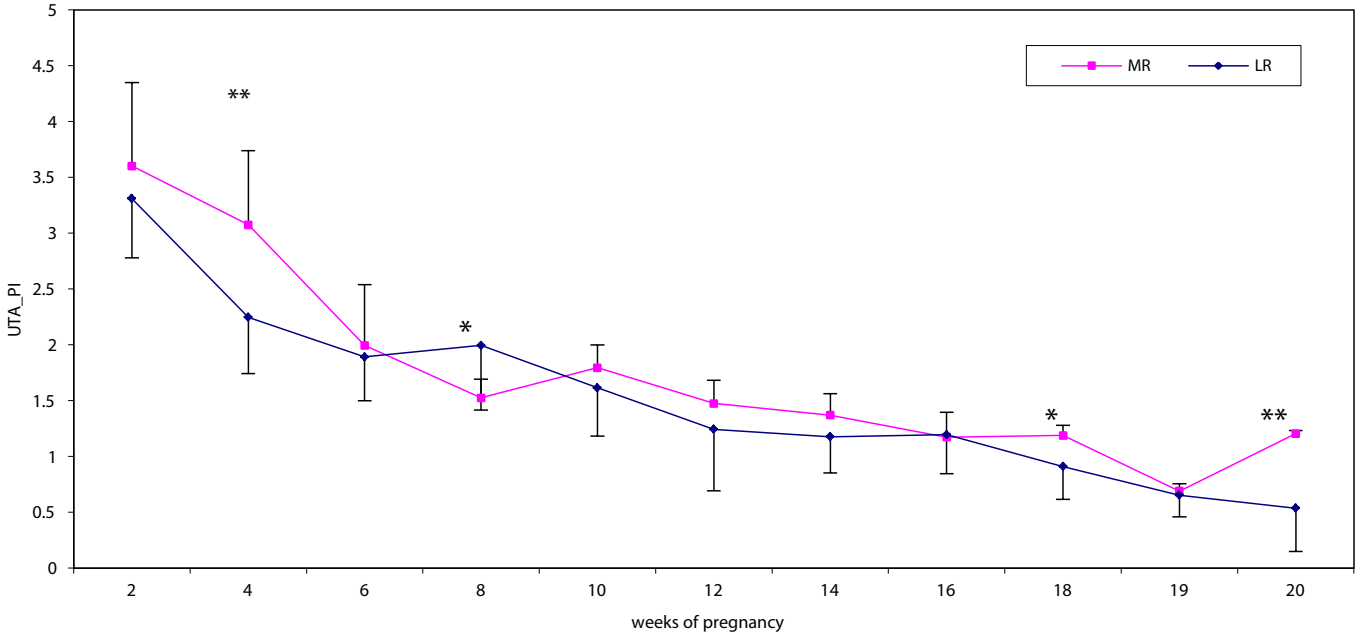
Umbilical artery: UMA-PI (P<0.02, week 8-20) and RI (P<0.01, week 8-20) were higher in MR than in LR goats. Perceptible differences of UMA- PI were obvious in fetuses of MR and LR does at wk 8, 10 and 14 (P<0.05-0.01, Figure 8) as well as wk 12, 14 and 16 for UMA- RI (P<0.05-0.001, Figure 9). Fetal heart rate was not significantly (P=0.81) different between LR and MR goats.

B-Mode measurements in goats: During the first eight weeks of pregnancy the diameter of the amniotic vesicle was significantly higher (P<0.01) in LR than in MR animals. From wk 8 until the end of gestation there was a significantly higher



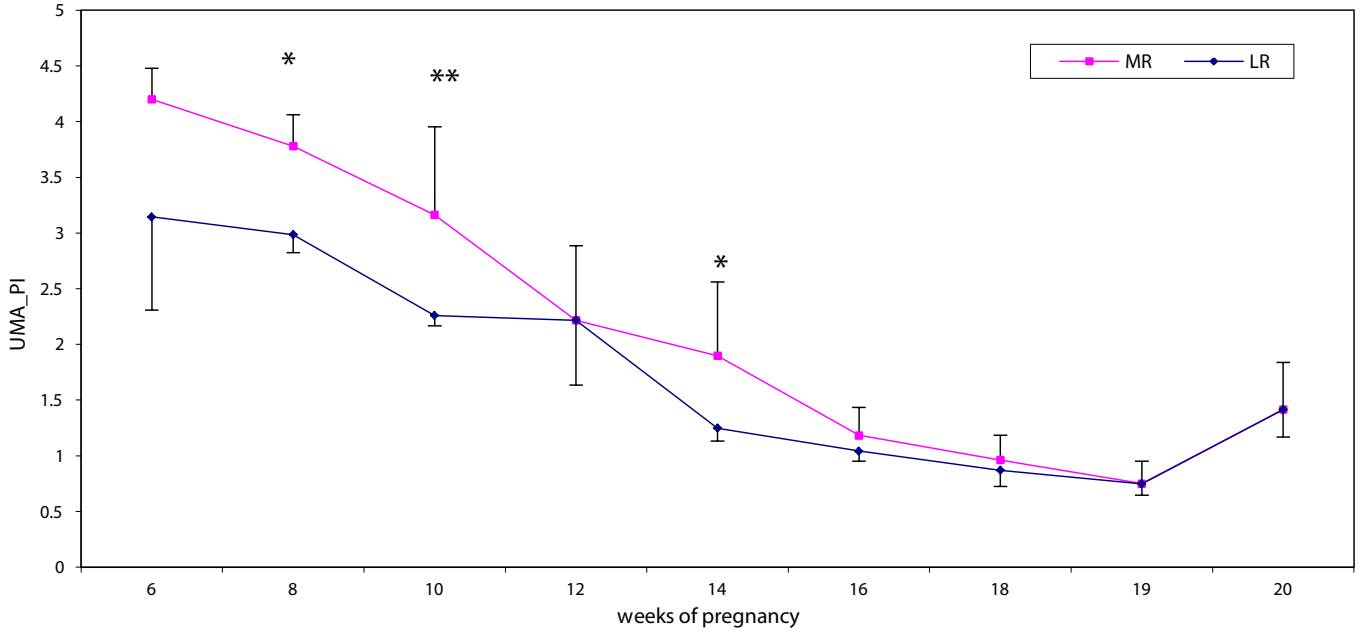
Mean ± SD of UTA-RI during pregnancy in MR (n=7 pregnancies) and LR (n=6545 pregnancies) goats. Values with asterisks differ between MR and LR goats at the same 546 pregnancy week (* P<0.05 and *** P<0.0001)

Figure 6: Effect of reactivity/anxiety on color Doppler parameters goats.



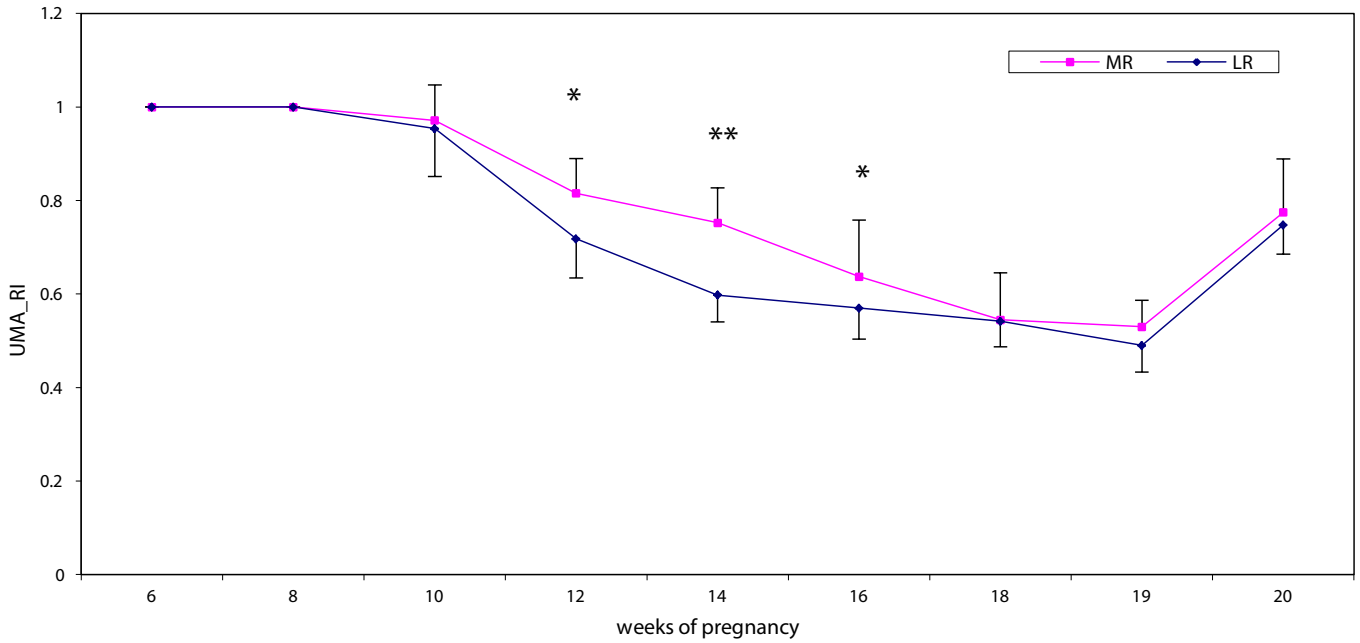
Values with asterisks differ between MR and LR goats at the same pregnancy week (* P<0.05 and ** P<0.01)

Figure 7: Mean ± SD of UTA-PI during pregnancy in MR (n=7 pregnancies) and LR (n=6 pregnancies) goats.



Values with asterisks differ between MR and LR goats at the same pregnancy week (* P<0.05 and ** P<0.01)

Figure 8: Mean ± SD of UMA-PI during pregnancy in MR (n=7 pregnancies) and LR (n=6 pregnancies) goats.



Values with asterisks differ between MR and LR goats at the same 561 pregnancy week (* P<0.05 and ** P<0.001)

Figure 9: Mean ± SD of UMA-RI during pregnancy in MR (n=7 pregnancies) and LR (n=6560 pregnancies) goats.

placentome diameter (P<0.005), fetal chest diameter (P<0.05) and a higher fetal metacarpal length (P<0.01) in LR than MR animals, while a tendency was recognized for a larger umbilical cord diameter (P<0.06) as well as for the biparietal head width (P<0.09) in LR versus MR animals. With regard to the fetal biometrical changes during pregnancy, a linear increase in fetal parameters, including biparietal head width, orbital diameter, occipito-snout length, chest diameter, umbilical cord diameter, fetal metacarpal length and placentome diameter occurred between wk 6 and 18 of pregnancy (P<0.0001).

Discussion

The use of non-invasive B-mode and Doppler ultrasound either to follow intrauterine fetal development and/or uterine blood flow changes during pregnancy in women has been reported.[13,17] In some studies an effect of maternal anxiety on Doppler and fetometric parameters has been recognized.[20,36]

Since this topic has not been addressed in animals, the aim of the present work was to evaluate the effect of the dams' temperament on fetal growth as well as on the maternal and umbilical Doppler parameters.

Non-invasive color Doppler sonography was not used to evaluate the uterine as well as the umbildams' temperament on fetal growth as well as on the maternal and umbilical Doppler parameters.

Non-invasive color Doppler sonography was not used to evaluate the uterine as well as the umbilical blood flow changes during normal pregnancy in sheep and goats.[28]

The present study revealed that the UtA-PI in ewes as well as the UtA-RI in goats was higher in MR than LR animals between wk 8-20 of pregnancy. Similar results were obtained in women. Teixeira et al. recognized an increase of UtA-RI in anxious pregnant women, and Vythilingum et al. mentioned a significant increase of UTA-PI before wk 21 of pregnancy anxious women.[17,18] But in the same study the anxiety score was not related to UtA-RI at wk 22 of gestation. On the other hand Mendelson et al. found no association between UTA-RI and UMA-RI and maternal distress.[37] The increased Doppler indices in the present study might be attributed to different factors discussed in the international literature. These include increased level of stress hormones; and transient changes in maternal hormone concentrations that affect uterine blood flow parameters.[17,20]

Higher values for PI and RI in MR than LR pregnant sheep and goats were recognized in the present study. Those results might be attributed to changes in level of cortisol, which was discussed especially in high anxiety score women.[20] Other data concerning changes in uterine and umbilical blood flow velocity, especially blood flow impedance indices, support the results obtained in the present study, too.[38-41] Furthermore, lower RI and PI values in uterine and umbilical arteries were associated with increased blood flow volume, attributed to vasodilatation of uterine vessels in combination with placental derived growth factors and local production of estrogens (and other steroids) from the placenta of ewes.[42,43]

In the present study no significant relationship between the fetal heart rate and dam's reactivity was recognized (P=0.46 and 0.81 in ewes and goats resp.). These data are consistent with previous results in human beings as well as in cattle with high risk pregnancies due to maternal diseases .[17,44-46] The non-significant results might be related to the small experimental numbers per groups.[45,46]

Intrauterine fetal growth depends on many factors including maternal, fetal, placental and genetically predetermined growth potential.[47] Already during the first 8 wk of pregnancy an increased diameter of the amniotic vesicle was seen in LR than MR sheep and goats. Later, from wk 8 until the end of pregnancy the fetal chests as well as the umbilical cord diameter were significantly larger in LR ewes. Also, in pregnant goats the placentomes and fetal chest diameter, as well as the fetal metacarpal length was significantly (P<0.005-0.05) larger in LR than MR animals. There was also a tendency towards an increased BPD and UMD (P>0.05). In humans similar results related to the head circumference, width and femur length have been reported.[13] Total birth weight of lambs as well as kids was significantly higher in LR than in MR sheep and goats in this study. These results were similar to that from Diego et al. and Henrichs et al. in maternally distressed humans as well as in ewes after artificial intrauterine growth restriction.[48-50] In contrast; Roussel et al. reported an increased birth weight of stressed lambs.[51]

Because of the results obtained after B-mode and Doppler investigations, it is concluded that changes in uterine and/or umbilical blood flow might have an important role in intrauterine fetal growth. Changes can be successfully imaged non-invasively, in order to demonstrate the effects of maternal anxiety in sheep as experimental model for human being.

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