

# Uterine perfusion and early pregnancy rates in brood mares

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## Summary

There are many studies about the effect of uterine blood flow on fertility in women, but to date no such information is available on mares. Therefore, we aimed to examine possible effects of uterine perfusion on early pregnancy rates in mares. Thirty five mares with a mean age of  $14.2 \pm 3.5$  yrs were investigated on Days -2 and -1 (0 = ovulation). Five of the mares were maiden, 23 barren and 7 mares were examined within the first two estrous cycles after birth (= foaling mares). All mares received 1.500 IU hCG i.v. on Day -2 at a mean follicle diameter of about 40 mm. Inseminations were carried out twice on Days -1 and 0 using cooled semen suspended in skim milk extender from fertile stallions. Uterine blood flow was studied transrectally by examining the uterine artery ipsilateral to the pre-ovulatory follicle using a colour Doppler ultrasonograph equipped with a 7.0 MHz microconvex-probe. Blood flow was expressed as blood flow volume (BFV). Pregnancy diagnosis was carried out sonographically in B-mode between Days 14 and 16. As there were no differences ( $P > 0.05$ ) in BFV values between Days -2 and -1 mean values were used for further evaluations. BFV ranged from 46.9 to 332.9 ml/min (mean  $\pm$  SD:  $181.0 \pm 76.8$  ml/min). BFV was higher ( $P < 0.02$ ) in foaling mares (mean  $\pm$  SD:  $249.1 \pm 67.4$  ml/min) than in barren (mean  $\pm$  SD:  $167.6 \pm 69.0$  ml/min) and maiden (mean  $\pm$  SD:  $147.3 \pm 81.4$  ml/min) mares. No differences ( $P = 0.56$ ) in BFV could be observed between barren and maiden mares. In 24 (68.6%) mares a conceptus could be detected during early pregnancy, while 11 (31.4%) mares were not pregnant at this time. Pregnant maiden mares ( $n = 3$  mares; mean:  $194.5$  ml/min) showed in tendency ( $P = 0.08$ ) higher BFV values compared to non-pregnant mares ( $n = 2$  mares; mean:  $76.5$  ml/min). In barren (Diff.:  $P = 0.97$ ) and foaling mares (Diff.:  $P = 0.34$ ) no differences in BFV values could be measured between pregnant and non-pregnant animals. In conclusion, the present study indicates that uterine blood flow during the pre-ovulatory period is not a limiting factor for fertilization and early pregnancy rates in uni- and multiparous mares, but in maiden mares. Investigations on a higher number of maiden mares are required to prove these results.

**Keywords:** uterine artery, Doppler sonography, mare, early pregnancy, fertility

## Uterine Durchblutung und Frühgraviditätsrate bei Zuchtstuten

Während in der Humangynäkologie eine Vielzahl von Studien über Zusammenhänge zwischen der uterinen Perfusion und der Fruchtbarkeit der Frau vorliegen, gibt es bisher keine derartigen Informationen für die Stute. Daher war das Ziel der vorliegenden Studie, mögliche Zusammenhänge zwischen dem uterinen Blutfluss und den Konzeptionsergebnissen bei der Stute zu untersuchen. Fünfunddreißig Stuten mit einem mittleren Alter von  $14,2 \pm 3,5$  Jahren wurden an den Zyklustagen -2 und -1 (0 = Ovulation) dopplersonographisch untersucht. Fünf der Stuten waren Maidenstuten, 23 güste Tiere und 7 der Stuten wurden innerhalb der ersten beiden Zyklen nach der Abfohlung untersucht (= Fohlenstuten). Allen Stuten wurden am Tag -2 bei einem Follikeldurchmesser von mindestens 40 mm 1.500 IE hCG intravenös verabreicht. Besamungen wurden an den Tagen -1 und 0 mit in Magermilchverdünner konserviertem Frischsperma von fertilen Hengsten durchgeführt. Der uterine Blutfluss wurde transrektal an der ipsilateral zum präovulatorischen Follikel gelegenen A. uterina gemessen und das Blutflussvolumen (BFV) zur Charakterisierung der uterinen Perfusionsverhältnisse bestimmt. Trächtigkeitsuntersuchungen wurden zwischen dem 14. und 16. Tag post ovulationem sonographisch im B-Mode durchgeführt. Da zwischen den Tagen -2 und -1 keine Unterschiede ( $p > 0,05$ ) in den BFV - Werten bestanden, wurden für die weiteren Auswertungen jeweils die Mittelwerte beider Tage herangezogen. Das uterine Blutflussvolumen variierte mit Werten von 46,9 bis 332,9 ml/min ( $x \pm s$ :  $181,0 \pm 76,8$  ml/min) sehr deutlich zwischen den Stuten. Fohlenstuten ( $x \pm s$ :  $249,1 \pm 67,4$  ml/min) zeigten gegenüber güsten Tieren ( $x \pm s$ :  $167,6 \pm 69,0$  ml/min) sowie Maidenstuten ( $x \pm s$ :  $147,3 \pm 81,4$  ml/min) ein höheres uterines Blutflussvolumen ( $p < 0,05$ ). Zwischen güsten Stuten und Maidenstuten waren keine Unterschiede ( $p = 0,56$ ) in den BFV-Werten festzustellen. Bei 24 (68,6%) der 35 farbdopplersonographisch untersuchten Stuten war während der Frühgravidität ein Konzeptus nachweisbar. Die übrigen 11 Stuten (31,4%) wurden in diesem Zeitraum als nicht tragend diagnostiziert. Trächtig gewordene Maidenstuten wiesen tendenziell (Diff.:  $p = 0,08$ ) ein höheres uterines Blutflussvolumen ( $n = 3$  Stuten;  $x$ :  $194,5$  ml/min) auf als leer gebliebene Maidenstuten. ( $n = 2$  Stuten;  $x$ :  $76,5$  ml/min). Bei güsten- (Diff.:  $p = 0,97$ ) und Fohlenstuten (Diff.:  $p = 0,34$ ) waren keine Unterschiede in den BFV Werten zwischen trächtig und nicht-trächtig gewordenen Tieren zu verzeichnen. Zusammenfassend gibt die vorliegende Studie Hinweise darauf, dass die uterine Blutversorgung bei Maidenstuten, aber nicht bei uni- und multiparen Tieren einen begrenzenden Faktor hinsichtlich Fertilisation und Frühträchtigkeitsrate darstellt. Diese Ergebnisse müssen jedoch noch an einer größeren Anzahl von Tieren überprüft werden.

**Schlüsselwörter:** Reproduktion, A. uterina, Doppler-Ultraschall, Stute, Frühgravidität, Fertilität

## Introduction

In human gynaecology Doppler ultrasonography is a well-established technique to monitor risk pregnancies and to diagnose fertility disorders in women (Goswamy and Steptoe 1988, Goswamy et al. 1988, Steer et al. 1992, Pattinson

1994). In the last few years this technique has been applied in mares, too (Bollwein et al. 1998). Results of these examinations indicated a relationship between resistance to uterine blood flow and fibrotic changes of the endometrium (Stolla und Bollwein 1997, Blaich 1999). Schoon et al. (1999) noti-

ced also in histological studies that pathological alterations of the endometrial glands and vessels are often associated. In newer investigations (Blaich et al. 2001, Ludwig et al. 2002) positive relationships between resistance to uterine blood flow and the degree of degenerative alterations of uterine vessels could be demonstrated. In all the above mentioned studies the resistance to blood flow in the uterine artery was used as an indicator for a poor uterine perfusion, but no measurements of uterine blood flow volume itself were performed. Furthermore, no studies have been carried out comparing uterine blood flow parameters with fertility rates after insemination of brood mares. Therefore, the aim of the present study was to prove whether uterine perfusion during the pre-ovulatory period has an effect on early pregnancy rates in brood mares by determining the blood flow volume.

## Material and Methods

Examinations were carried out on 35 Hanoverian mares with a mean age of 14.2 years (range, 10 to 22 years). Five of the mares were maiden, 7 foaling and 23 barren. The foaling mares were examined during the first or second estrus after birth. Only mares showing negative results of cytological and bacteriological examinations of uterine swabs taken during estrus were included in the study. In addition, only estrous cycles with an ovulation occurring between 36 and 48 hours after i.v. injection of hCG were used for further evaluations (see below).

Mares showing clinical signs of estrus, a distinct endometrial oedema and a pre-ovulatory follicle with a mean diameter of at least 40 mm were given 1.500 IU hCG i.v.. Twelve and 36 hours later the mares were inseminated twice with cooled semen from fertile Hanoverian warmblood stallions containing  $300 \times 10^6$  progressively motile sperm. Pregnancy diagnosis was performed between Days 14 and 16 using B-mode sonography.

Uterine blood flow was measured on Days -2 and -1 (0 = day of ovulation) using transrectal colour Doppler sonography. As in a previous study (Diaz-Peinado 2003) no differences and high correlations in BFV values between uterine arteries of both sides were noticed, only the uterine artery ipsilateral to the pre-ovulatory follicle was examined. Using the Eccocee CX (Toshiba, Tokyo, Japan) and a 7.0 MHz micro-convex probe, uterine blood flow volume was calculated by measuring the time averaged maximum velocity (TAMV) and the diameter of the uterine artery (D) as described elsewhere (Bollwein et al. 2002).

Statistical analyses were carried out using the View 5.0 statistical software package (SAS Institute Inc., Cary / North Carolina, 1998). Measurements were subjected to analysis of variance. In addition, Fisher's protected LSD was conducted to determine differences in BFV between maiden, barren and foaling mares and Mann Whitney test was used to prove whether there are differences in BFV between pregnant and non-pregnant animals.

## Results

As there were no differences in blood flow parameters between Days -2 and -1 ( $P > 0.05$ ), mean values were used for further analyses.

There were high variabilities in all blood flow parameters. TAMV ranged from 9.2 to 37.1 (mean  $\pm$  SD:  $17.4 \pm 6.2$  cm/sec) and D from 2.3 to 6.9 (mean  $\pm$  SD:  $4.7 \pm 1.1$  mm). BFV values showed a minimum of 46,9 ml/min and a maximum of 332,9 ml/min (mean  $\pm$  SD:  $181.0 \pm 76.8$  ml/min). TAMV was higher ( $P < 0.004$ ) in maiden than in barren and foaling mares (Tab. 1). Between barren and foaling mares no differences ( $P = 0.73$ ) in TAMV values occurred. Maiden mares showed the smallest ( $P < 0.006$ ), and foaling mares the biggest ( $P < 0.006$ ) vessel diameter. The highest BFV values were observed in foaling mares ( $P < 0.02$ ). No differences ( $P = 0.56$ ) in BFV were calculated between barren and maiden mares (Tab. 1).

**Tab 1** Time averaged maximum velocity (TAMV), diameter (D) and blood flow volume (BFV) of the uterine artery ipsilateral to the preovulatory follicle in maiden, barren and foaling mares. Values are means  $\pm$  SD of Days 2 and 1 (0 = ovulation).  
*Zeitlich gemittelte maximale Blutflussgeschwindigkeit (TAMV), Durchmesser (D) und Blutflussvolumen (BFV) der ipsilateral zum präovulatorischen Follikel gelegenen A. uterina bei Maiden-, güsten und Fohlenstuten. Die Werte sind Mittelwerte  $\pm$  s der Tage -2 und -1 (O=Ovulation).*

Reproductive status	n (Mares)	TAMV [cm/s]	D [mm]	BFV [ml/min]
Maiden	5	25,9 $\pm$ 9,1 <sup>a</sup>	3,4 $\pm$ 1,0 <sup>a</sup>	147,3 $\pm$ 81,4 <sup>a</sup>
Barren	23	15,8 $\pm$ 4,0 <sup>b</sup>	4,7 $\pm$ 0,8 <sup>b</sup>	167,6 $\pm$ 69,0 <sup>a</sup>
Foaling	7	16,5 $\pm$ 5,7 <sup>b</sup>	5,8 $\pm$ 1,1 <sup>c</sup>	249,1 $\pm$ 67,4 <sup>b</sup>

<sup>a,b</sup> values with different superscripts differ significantly ( $P < 0,05$ )

A conceptus was detectable between Days 14 and 16 in 24 (68,8 %) of the mares, while the remaining 11 (31,4 %) mares were diagnosed as non-pregnant at this time (Tab. 2). Mean BFV values were 2.5 fold higher in pregnant compared to non-pregnant maiden mares. This difference was based on the diameter of the uterine vessel in pregnant mares, which was about twice as high as in non-pregnant maiden mares. Since the group of maiden mares consisted only of 5 animals, BFV- values differed only in tendency ( $P = 0.08$ ). No associations between blood flow parameters and pregnancy results were measurable in barren and foaling mares.

## Discussion

The present study indicates that variabilities in uterine blood flow volume are mainly caused by status of parity in mares. Animals, which were examined within the first two estrous cycles after birth showed the highest uterine perfusion based on an increased diameter of the uterine artery. In another Doppler sonographic investigation (Woschée 1999) performed during pregnancy in mares it was shown, that the diameter of the uterine vessels shows a 5 fold rise from 3 to 15 mm during the course of gestation. The diameter measured in the present study in foaling mares was about twice as high as the diameter observed in the above mentioned investigations during early pregnancy. The comparison of the results of both studies leads to the conclusion that the involution of the uterine vessels is not completed within the first two estrous

cycles after birth. Even in uni- or multiparous mares, which were barren for at least one year, the uterine vessel diameter was bigger compared to maiden mares. These differences, however, did not lead to a higher blood perfusion in barren mares, because maiden mares showed a higher blood flow velocity than barren mares. The reason for the variability in blood flow velocity between nulli- and uni-/multiparous mares is not clear, but presumably it is caused by some regulatory mechanisms of the circulatory system. Assuming a nearly consistent cardiac output and blood pressure in nulli- and uni-/multiparous mares, the uterine blood flow volume could be regulated in part by a higher blood flow velocity in mares with smaller vessel and vice versa.

**Tab 2** Time averaged maximum velocity (TAMV), diameter (D) and blood flow volume (BFV) of the uterine artery ipsilateral to the preovulatory follicle in maiden, barren and foaling mares. Values are means of Days 2 and 1 (0 = ovulation).  
*Zeitlich gemittelte maximale Blutflussgeschwindigkeit (TAMV), Durchmesser (D) und Blutflussvolumen (BFV) der ipsilateral zum präovulatorischen Follikel gelegenen A. uterina bei trächtig und nicht-trächtig gewordenen Maiden-, güsten und Fohlenstuten. Die Werte sind Mittelwerte der Tage -2 und -1 (O=Ovulation)*

Reproductive status	n (Mares)	TAMV [cm/s]	D [mm]	BFV [ml/min]
Pregnant maiden	3	24,9	4,1 <sup>a</sup>	194,5 <sup>a</sup>
Non pregnant maiden	2	27,5	2,4 <sup>b</sup>	76,5 <sup>b</sup>
Pregnant barren	18	15,5	4,7	167,9
Non pregnant barren mares	5	16,8	4,6	166,6
Pregnant foaling	3	14,0	6,6	279,8
Non-Pregnant foaling	4	18,5	5,2	226,1

<sup>a,b</sup> corresponding values with different superscripts in columns differ in tendency (P = 0.08)

The most interesting result of the present study was the low uterine blood flow volume observed in maiden mares, which did not get pregnant (Tab. 2). The vessel diameter in these animals was only about half as big as in non-pregnant mares. It is well known that a relatively high number of older mares is subfertile, but to the best of our knowledge there are no explanations for these differences. The data of this study suggest that in some of the maiden mares a reduced blood flow volume because of a small vessel diameter may be a reason for subfertility. However, this assumption is only based on measurements in a small group of mares. Much more maiden mares have to be investigated to prove this hypothesis. No relationships between uterine blood flow volume and early pregnancy rates could be observed in uni- and multiparous mares. As already mentioned above, these mares showed a

higher diameter of uterine vessels because of the vasodilation during previous pregnancies.

In summary, the results show that uterine blood flow volume is higher in foaling than in barren and maiden mares. It may be a limiting factor for fertilization and early pregnancy in some maiden-, but not in uni- and multiparous mares.

## Literature

- Blaich U. (1999): Dopplerultrasonographische Untersuchungen zur uterinen Hämodynamik des Pferdes unter Berücksichtigung sexualzyklischer und degenerativ-endometrialer Einflüsse. Hannover, Tierärztl. Hochsch., Diss.
- Blaich U., Bartmann C. P., Schoon D., Klug E. and Schoon H.-A. (2001): Comparative Doppler sonographic and pathohistologic investigations of the uterine artery in the horse. *Pferdeheilkunde* 17, 687
- Bollwein H., Maierl H., Mayer R. and Stolla R. (1998): Transrectal color Doppler sonography of the A. uterina in cyclic mares. *Theriogenology* 49, 1483-1488
- Bollwein H., Baumgartner U. and Stolla R. (2002): Transrectal Doppler sonography of uterine blood flow in cows during pregnancy. *Theriogenology* 57, 2053-2061
- Diaz-Peinado L. (2003): Zusammenhänge zwischen der genitalen Durchblutung und der Fertilität bei der Stute. Univ. München, Gynäkologische und Ambulatorische Tierklinik., Diss.
- Goswamy R. K. and Steptoe P. C. (1988): Doppler ultrasound studies of the uterine artery in spontaneous ovarian cycles. *Hum. Reprod.* 3, 721-726
- Goswamy R. K., Williams G. and Steptoe P. C. (1988): Decreased uterine perfusion - a cause of infertility. *Hum. Reprod.* 3, 955-959
- Ludwig S., Blaich U., Schoon D., Aupperle H., Schoon H.-A., Bartmann C. P. and Klug E. (2002): Functional pathology of the uterine blood flow in the mare – pathohistologic and Doppler sonographic investigations. *Theriogenology* 58, 511-513
- Pattinson R. C., Norman K. and Odendaal H. (1994): The role of Doppler velocimetry in the management of high risk pregnancies. *Br. J. Obstet. Gynaecol.* 101, 114-120
- Schoon D., Schoon H.-A. and Klug E. (1999): Angioses in the equine endometrium – Pathogenesis and clinical correlations. *Pferdeheilkunde* 15, 541-546
- Steer C. V., Campbell S., Tan S. L., Crayford T., Mills C., Mason B.A. and Collins W. P. (1992): The use of transvaginal color flow imaging after in vitro fertilization to identify optimum conditions before embryo transfer. *Fertil. Steril.* 57, 372-376
- Stolla R. and Bollwein H. (1997): Color Doppler sonography of the uterine artery in subfertile mares. *Pferdeheilkunde* 13, 547
- Woschée I. (1999): Farbdopplersonographische Untersuchung der Ateria uterina, Ateria ovarica und Ateria umbilicalis während der Gravidität und des Puerperiums der Stute. Univ. München, Gynäkologische und Ambulatorische Tierklinik., Diss.

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