Application of Doppler ultrasonography on genital organs

Clinic for cattle
University of Veterinary Medicine Hannover, Germany

www.tiho-hannover.de
- Colour Doppler sonography extended the scope of sono-graphic imaging from a morphologic to a **physiologic** basis.

- Since more than two decades Colour Doppler sonography has been used in **human medicine** for the evaluation of uterine and fetal blood supply in women undergoing an **embryo-transfer** and in patients with **risk pregnancies**.

- Until recently in cows genital blood supply could only be measured by using **surgically implanted** blood flow probes.
Aims

- Development of non-invasive techniques for the determination of genital blood flow by using transrectal Doppler sonography

- Examination of genital blood flow under physiological and pathological conditions

- Investigation of the effects of different treatments on genital blood flow
Outline

- Basics and technique of colour Doppler sonography
- Examinations of uterine blood flow
- Examinations of ovarian blood flow
Outline

- Basics and technique of colour Doppler sonography

- Examinations of uterine blood flow

- Examinations of ovarian blood flow
Basics of Doppler-Sonography

Reflection of ultrasound waves by moving blood cells

Frequency shift

Blood flow velocity
Frequency Shift in Colour Mode
Frequency Shift in Spectral Mode

Time [s]

Frequency shift [Hz]

systole

diastole
Changes in blood flow velocity during time

Time averaged maximum blood flow velocity
Quantification of resistance to blood flow using the resistance (RI) and Pulsatility Index (PI)

$RI = \frac{S-D}{TAMV}$

$PI = \frac{S-M}{TAMV}$

S: maximum systolic frequency shift
M: minimum diastolic frequency shift
D: enddiastolic frequency shift
TAMV: time averaged maximum frequency shift
Quantification of blood flow using the blood flow volume (BFV)

$$BFV = A \times V$$

A: cross-sectional area of the vessel
V: blood flow velocity
Color Doppler sonography of uterine blood flow
Colour Doppler Sonography
of a preovulatory follicle of a cow in Power Mode
Colour Doppler Sonography of the Corpus luteum of a mare in Power Mode
Colour Doppler Sonography of the Corpus luteum of a cow in Power Mode
Quantification of luteal blood flow

Pixelflux
(Chameleon-Software, Leipzig, Germany)

ACL pixel: area of colour pixels (cm²)
ACLtotal: maximal cross section area (cm²)
Colour Doppler sonography machines
7.0 MHz Microconvex probe
Technique of transrectal colour Doppler Sonography

- Ovarian aretry
- Uterine aretry
Transrectal colour Doppler sonography of genital blood flow in large animals
Outline

- Basics and technique of colour Doppler sonography

- Examinations of uterine blood flow

- Examinations of ovarian blood flow
Examinations of uterine blood flow

- Alterations during estrous cycle, after insemination, during pregnancy and after parturition

- Differences between mares and relationships between uterine blood flow and fertility

- Effects of exogenous sexual steroids, vasodilating and anticoagulative drugs on uterine perfusion
Examinations of uterine blood flow

- Alterations during estrous cycle, after insemination, during pregnancy and after parturition

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Resistance to uterine blood flow (PI) and progesterone (P) in mares
Resistance to uterine blood flow (PI) and estrogens (E) in mares
Changes in uterine blood flow velocity, plasma estrogens and gene expression of estrogen receptors in mares

Day of estrous cycle

<table>
<thead>
<tr>
<th>Day</th>
<th>TAMV</th>
<th>Total plasma estrogens</th>
<th>mRNA ER α</th>
<th>mRNA ER β</th>
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<tbody>
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</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td>b</td>
</tr>
<tr>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td>d</td>
</tr>
</tbody>
</table>

- TAMV = Total Plasma Monocyte Volume
- mRNA ER α = mRNA Estrogen Receptor α
- mRNA ER β = mRNA Estrogen Receptor β
Changes in uterine blood flow velocity, plasma nitrate/nitrite and gene expression of eNOS and iNOS in mares

Relative changes [%]

Day of estrous cycle

0 1 5 11 15 21

0 25 50 75 100 125 150 175 200 225 250

= TAMV = Plasma NO_{2}/NO_{3}

= mRNA eNOS = mRNA iNOS

Changes in uterine blood flow velocity, plasma nitrate/nitrite and gene expression of eNOS and iNOS in mares
Examinations of uterine blood flow

- Alterations during estrous cycle, after insemination, during pregnancy and after parturition

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- Effects of exogenous sexual steroids, vasodilating and anticoagulative drugs on uterine perfusion
Changes of uterine blood flow velocity (TAMV) after intrauterine infusion of raw semen and seminal plasma

- Old mares, raw semen
- Young mares, raw semen
- Old mares, seminal plasma
- Young mares, seminal plasma
Changes of ovarian blood flow velocity (TAMV) after intrauterine infusion of raw semen and seminal plasma.

- Green bars: Dominant artery, raw semen
- Orange bars: Dominant artery, seminal plasma
- Yellow bars: Non-dominant artery, raw semen
- Green bars with yellow tips: Non-dominant artery, seminal plasma

Time after infusion in hours: 1, 3, 6, 12, 24.
Examinations of uterine blood flow

- Alterations during estrous cycle, after insemination, during pregnancy and after parturition

- Differences between mares and relationships between uterine blood flow and fertility

- Effects of exogenous sexual steroids, vasodilating and anticoagulative drugs on uterine perfusion
Uterine blood flow velocity (TAMV) during estrous cycle and early pregnancy in mares

![Graph showing uterine blood flow velocity (TAMV) during estrous cycle and early pregnancy in mares. The graph indicates a significant increase in TAMV from day 0 to day 21, with marked differences between the estrous cycle and early pregnancy phases. The data points are marked with asterisks for statistical significance.]
Uterine blood flow velocity (TAMV)
In the uterine arteries ipsi- and contralateral to the conceptus in mares

Day of pregnancy

TAMV [cm/s]
Uterine blood flow velocity before and after induction of embryonic mortality by using PGF$_{2\alpha}$
Colour Doppler sonography of the heart beats of the embryo / fetus
Alterations of frequency of heart beats of equine embryos/fetuses during pregnancy

Day of pregnancy

Heart beats per minute
Changes in resistance to uterine blood flow during pregnancy

Bollwein et al., 2004
Changes in diameter of the uterine vessels during pregnancy

Bollwein et al., 2004

healthy mares (4)

mare with abortion (1)
Changes in uterine blood flow volume during pregnancy

Bollwein et al., 2004

healthy mares (4)
mare with abortion (1)
Changes in resistance to umbilical blood flow during pregnancy

![Graph showing changes in resistance index during pregnancy. The graph depicts two lines, one for healthy mares (4) and one for a mare with abortion (1). The x-axis represents the week of pregnancy, ranging from 20 to 46. The y-axis represents the resistance index, ranging from 0.45 to 0.80. The graph illustrates a decrease in resistance index over time, with the line for healthy mares showing a more consistent decrease compared to the line for the mare with abortion.](image-url)

Bollwein et al., 2004
Examinations of uterine blood flow

- Alterations during estrous cycle, after insemination, during pregnancy and **after parturition**

- Differences between mares and relationships between uterine blood flow and fertility

- Effects of exogenous sexual steroids, vasodilating and anticoagulative drugs on uterine perfusion
Changes in uterine size and weight during the first three weeks after parturition in cows

(P. Sengen, 2005)
Macroscopic uterine involution during the first three weeks after parturition in cows

Day of birth

Uterine blood flow volume (BFV) and resistance to uterine blood flow (PI) in cows

Values with different letters differ between days ($P \leq 0.05$)
Uterine blood flow volume (BFV) in healthy cows and in cows with a metritis during the first two weeks after parturition.

- Healthy cows (n = 4)
- Cows with metritis (n = 6)

Significance: $P < 0.05$
Resistance to uterine blood flow (PI) in healthy cows and in cows with a metritis during the first two weeks after parturition

- Healthy cows (n = 4)
- Cows with metritis (n = 6)

$P < 0.05$
Conclusions

- There are characteristic changes in uterine and blood flow during estrous cycle, after insemination, during pregnancy and after parturition.

- The reasons for these alterations are not completey known.
Examinations of uterine blood flow

- Alterations during estrous cycle, after insemination, during pregnancy and after parturition

- Differences between mares and relationships between uterine blood flow and fertility

- Effects of exogenous sexual steroids, vasodilating and anticoagulative drugs on uterine perfusion
Variations in uterine blood flow and their effect on early pregnancy rates in brood mares

Total Number: 35

Age:
- range [years]: 10 - 22
- mean ± SD [years]: 14.2 ± 3.5

Reproductive Status:
- Maiden: 5
- Barren: 23
- Lactating: 7
Result of early pregnancy diagnosis

Total pregnant mares: 24 (69 %)

Pregnancy rate and reproductive status:

Maiden 3 (60 %)
Barren 18 (78 %)
Lactating 4 (75 %)
Blood flow volume (BFV) and time averaged maximum velocity (TAMV)

$r = 0.24; P > 0.05$
Blood flow volume (BFV) and diameter (D) of uterine arteries

\[ r = 0.74; \ P < 0.0001 \]
Blood flow volume (BFV) and reproductive status

![Bar graph showing blood flow volume (BFV) for lactating, barren, and maiden states.]

- **Lactating** (n = 7): BFV ~ 250 mL/min
- **Barren** (n = 23): BFV ~ 150 mL/min
- **Maiden** (n = 5): BFV ~ 100 mL/min

Legend:
- a: Significant difference
- b: Non-significant difference
Blood flow volume (BFV) and result of early pregnancy diagnosis

![Bar graph showing BFV in pregnant and non-pregnant individuals.](image)

- Pregnant: n = 24
- Non-Pregnant: n = 11
Blood flow volume (BFV), reproductive status and result of early pregnancy diagnosis

- Lactating Maiden:
  - BFV (mL/min): n = 4

- Barren:
  - BFV (mL/min): n = 3, P = 18

- Maiden:
  - BFV (mL/min): n = 3

- Barren:
  - BFV (mL/min): n = 5

- Maiden:
  - BFV (mL/min): n = 2

- P = pregnant
- NP = non pregnant
Diameter of the uterine artery, reproductive status and result of early pregnancy diagnosis

- **P** = Pregnant
- **NP** = Non-Pregnant

<table>
<thead>
<tr>
<th>Status</th>
<th>Pregnant (P)</th>
<th>Non-Pregnant (NP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactating</td>
<td>n = 4</td>
<td>n = 3</td>
</tr>
<tr>
<td>Barren</td>
<td>n = 18</td>
<td>n = 5</td>
</tr>
<tr>
<td>Maiden</td>
<td>n = 3</td>
<td>n = 2</td>
</tr>
</tbody>
</table>
Uterine blood flow volume and fertility of mares

Volume (ml/min)

-2 -1 0 1 2 4 6 8 10 12

Insemination

Day of estrous cycle / pregnancy

- pregnant
- barren

* indicates significant differences
# indicates significant differences compared to the day of insemination
Conclusions

- Differences in uterine blood flow volume between mares are mainly caused by variations in diameter of the arteries.

- Uterine blood flow volume is not a limiting factor for early pregnancy in uni-/multiparous mares.

- Uterine blood flow volume may be a limiting factor for early pregnancy in some maiden mares.

- An increase in uterine blood flow may an indicator for an increased inflammatory response in subfertile mares.
Examinations of uterine blood flow

- Alterations during estrous cycle, after insemination, during pregnancy and after parturition

- Differences between mares and relationships between uterine blood flow and fertility

- Effects of exogenous sexual steroids, vasodilating and anticoagulative drugs on uterine perfusion
Effect of altrenogest on resistance to uterine blood flow (PI)

- Control group
- Altrenogest
Effect of estradiol benzoate on resistance to uterine blood flow (PI)
Effect of vasodilatatory and anticoagulatory drugs on uterine blood flow (PI) in mares

![Graph showing the effect of different drugs on uterine blood flow (PI) over the course of the estrous cycle.](image)

- **Placebo**
- **Acetyl salicylic acid**
- **Captopril**
- **Isosorbide dinitrate**

Day of estrous cycle:

- 11
- 10
- 9
- 8
- 7
- 6
- 5
- 4
- 3
- 2
- 1
Outline

- Basics and technique of colour Doppler sonography

- Examinations of uterine blood flow

- Examinations of ovarian blood flow
Examinations of ovarian blood flow in cows

- Changes in luteal blood flow during estrous cycle and early pregnancy

- Effects of vasodilating and anticoagulative drugs on ovarian perfusion and progesterone values

- Effect of hormonal treatments
Examinations of ovarian blood flow in cows

- Changes in luteal blood flow during estrous cycle and early pregnancy

- Effects of vasodilating and anticoagulative drugs on ovarian perfusion and progesterone values

- Effect of hormonal treatments
Plasma progesterone level, luteal size and luteal blood flow during estrous cycle in cows
Luteal blood flow in cycling, non pregnant and pregnant cows

Day of estrous cycle/pregnancy

Colour pixel area [cm²]

- Cyclic (n = 14)
- Non pregnant (n = 14)
- Pregnant (n = 18)
Examinations of ovarian blood flow in cows

- Changes in luteal blood flow during estrous cycle and early pregnancy

- Effects of vasodilating and anticoagulative drugs on ovarian perfusion and progesterone values

- Effect of hormonal treatments
Effect of vasodilatatory and anticoagulatory drugs on ovarian blood flow (PI) in mares
Effect of vasodilatatory and anticoagulatory drugs on plasma progesterone levels

Day of estrous cycle

Progesterone [nmol/ml]

0 20 40 60 80 100 120

1 2 3 4 5 6 7 8 9 10 11

- placebo
- captopril
- acetyl salicylic acid
- isosorbide dinitrate
Effects of vasodilatatory and anticoagulatory drugs on uterine blood flow and progesterone values

- Captopril
- ASA
- ISDN

- $\text{PI} = \text{PI of the ovarian artery}$
- $\text{Progesterone}$
Examinations of ovarian blood flow in cows

- Changes in luteal blood flow during estrous cycle and early pregnancy

- Effects of vasodilating and anticoagulative drugs on ovarian perfusion and progesterone values

- Effect of hormonal treatments
Effects of hCG on luteal blood flow and on plasma progesterone values

### Luteal blood flow

<table>
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<tr>
<th>Time [h]</th>
<th>NaCl</th>
<th>hCG</th>
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### Plasma progesterone

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<th>Time [h]</th>
<th>NaCl</th>
<th>hCG</th>
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<tr>
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<tr>
<td>48</td>
<td>0.0</td>
<td>0.0</td>
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</table>
Effects of a shortened oestrus on follicular and luteal blood flow in cows

1. GnRH 7d PGF2α 40h 2. GnRH 8d

Follicular blood flow

Luteal blood flow

Coloured Pixel area [cm²]

S  L

S  L
Effect of a shortened diestrous on follicular and uterine blood in cows

CDS = Colour Doppler sonography
Follicular size (FS), follicular blood flow (FBF), uterine blood flow (UTAMV) and estrogen levels during a spontaneous cycle and after PGF2α-induced luteolysis during the first and second follicular wave.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day</th>
<th>spontan. cycle (n=9)</th>
<th>1st wave cycle (n=9)</th>
<th>2nd wave cycle (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS [mm²]</td>
<td>-1</td>
<td>245 ± 8ᵃ</td>
<td>327 ± 16ᵇ</td>
<td>182 ± 13ᶜ</td>
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<tr>
<td>FS [mm²]</td>
<td>0</td>
<td>272 ± 13ᵃ</td>
<td>364 ± 15ᵇ</td>
<td>2.21 ± 9ᶜ</td>
</tr>
<tr>
<td>FBF [mm²]</td>
<td>-1</td>
<td>45 ± 4ᵃ</td>
<td>60 ± 5ᵇ</td>
<td>0.35 ± 6ᵃ</td>
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<tr>
<td>FBF [mm²]</td>
<td>0</td>
<td>58 ± 5ᵃ</td>
<td>98 ± 9ᵇ</td>
<td>0.54 ± 3ᵃ</td>
</tr>
<tr>
<td>UTAMV [cm/sec]</td>
<td>1</td>
<td>47.0 ± 1.25ᵃ</td>
<td>61.0 ± 4.36ᵇ</td>
<td>41.7 ± 2.68ᵃ</td>
</tr>
<tr>
<td>UTAMV [cm/sec]</td>
<td>0</td>
<td>43.4 ± 2.64</td>
<td>44.0 ± 4.39</td>
<td>40.8 ± 2.48</td>
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<tr>
<td>E_{tot} [pg/ml]</td>
<td>-1</td>
<td>51.9 ± 6.2ᵃᵇ</td>
<td>62.8 ± 8.9ᵇ</td>
<td>34.9 ± 9.0ᵃ</td>
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<tr>
<td>E_{tot} [pg/ml]</td>
<td>0</td>
<td>31.5 ± 5.5</td>
<td>26.6 ± 2.6</td>
<td>30.4 ± 5.5</td>
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</table>

Red marked values are higher (P<0.05) compared to corresponding values.
Conclusions

- Transrectal colour Doppler sonography is a useful method for the non-invasive investigation of genital blood flow in large animals.

- Transrectal colour Doppler sonography provides additional information about changes in genital blood flow caused by physiological and pathological conditions.

- By using colour Doppler sonography it is possible to investigate the effects of different treatments.
Thank you for your attention