Pferdeheilkunde – Equine Medicine 35 (2019) 1 (January/February) 11–19 DOI 10.21836/PEM20190102

Equine fetal gender determination in mid- and advanced-gestation by transabdominal approach – comparative study using 2D B-Mode ultrasound, Doppler sonography, 3D B-Mode and following tomographic ultrasound imaging

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Summary: Gender determination of the equine fetus is of big interest for the owner of a mare, particularly when planning the breeding purposes or due to economic reasons. This study aims to evaluate the feasibility of transabdominal 3D tomographic ultrasound imaging (TUI) as an additional diagnostic tool for gender determination. Special reference should be given to the hands-on experience of the examiner in the non-invasive transabdominal approach. Pregnancy checks were performed on 669 mares on various Thoroughbred stud farms in the mid-west of Germany in 2015 and 2016. Fetal sex was determined by 2D B-Mode ultrasound, 2D Doppler sonography and 3D imaging. Fetal gender was determined in a serial examination; time for each mare was limited to a maximum of 3 minutes. Predicted gender in 2015 and 2016 was compared to the gender at birth to determine accuracy of the methods. Transabdominal sonography was performed on 386 pregnant mares in 2015 and 283 mares in 2016. The gender of the fetus could be determined in 297 (~77%, year 2015) and 184 cases (~65%, year 2016) respectively, within the three-minute examination time frame. 3D imaging was realized in 118 (~40%, year 2015) and 94 cases (~51% year 2016) respectively. Combined transabdominal examination with B-Mode, Doppler and 3D TUI analysis led to high accuracies of correct gender diagnosis (~94% (2015) and ~96% (2016)). 3D TUI imaging allowed a gender diagnosis in 18 cases where B-Mode and Doppler sonography showed doubtful results (2015). 3D TUI of the fetal gonads was shown to be useful to increase the accuracy of gender determination in mares during mid- and advanced-gestation.

Keywords: mare, fetal gender determination, transabdominal sonography, fetal gonads, 3D ultrasound

Citation: Pricking S., Spilker K., Martinsson G., Rau J., Tönißen A., Bollwein H., Sieme H. (2019) Equine fetal gender determination in mid- and advanced-gestation by transabdominal approach – comparative study using 2D B-Mode ultrasound, Doppler sonography, 3D B-Mode and following tomographic ultrasound imaging. Pferdeheilkunde 35, 11–19; DOI 10.21836/PEM20190102

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Introduction

Gender diagnosis in the equine fetus has become more relevant in recent years mainly due to economic reasons (sale of a pregnant mare with a fetus of preferred sex; preferred sex in certain equestrian sport disciplines, future breeding purpose of a broodmare) (*Aurich & Schneider* 2014; *Bucca* 2011).

Fetal sexing in veterinary practice is mainly performed by ultrasound of the fetus, either transrectally at approximately 2 months or transabdominally as early as Day 90–100 of gestation with an optimal diagnostic window between Day 120 and 210. (*Curran & Ginther* 1989; *Renaudin* et al. 1997; *Bucca* 2005; *Tönissen* et al. 2015). Transrectal approach aims to identify the genital tubercle in early gestation, which is the forerunner of penis and clitoris. Previously published studies indicate that the genital tubercle can be best visualized between Day 59 and 68 of gestation. Major disadvantages of transrectal gender determination are the necessity of an experienced examiner and a restricted time frame for successful gender determination (*Renaudin* et al. 1997). *Kotoyori et al.* (2010; 2012) showed that 3D ultrasound allows identification of the genital tubercle between Day 63 and 76 as well as imaging of the external genitalia organs between Day 90 and 150 of gestation. The authors hypothesized that in the near future, 3D transrectal ultrasound would be more objective than conventional 2D ultrasound for diagnosing fetal gender.

Sex determination in mid and advanced gestation by identifying fetal primary sex organs is the preferable technique for gender diagnosis because of a larger diagnostic window (*Bucca* 2005). The fetus is staged in posterior presentation and a combined transrectal and transabdominal examination may be preferable to increase accuracy of gender determination between Day 90 and 150 (*Bucca* et al. 2005). Reaching the fetal hindquarters transrectally is almost impossible after 150 days of gestation and a transabdominal approach of gender determination is mandatory. Though transcutaneous transabdominal imaging is possible as early as Day 90–100 with an optimal diagnostic window of Day 120–210. Transabdominal ultrasound can be performed up to the end of pregnancy (*Bucca* 2005; *Schönbom* et al. 2015). The ultrasound beam is orientated towards the caudal fetal abdomen. Gonads can be visualized within the fetal caudal abdomen close to the kidney and the bladder. The structure of the gonads is evaluated, male gonads appear uniformly echodense, while differentiation of cortex and medulla (echodense core, hyperechogenic cycle) is possible in the female gonads (*Bucca* 2011). In male gonads homogenous echogenicity and a typical hyperechogenic line (representing mediastinum testis) along the longitudinal axis are characteristic (*Renaudin* et al. 1997). Typical B-Mode features can be even better visualized by using color Doppler ultrasonography (*Tönissen* et al. 2016). Doppler sonography shows an intense blood flow signal along the central line in the male fetus (representing blood flow in mediastinum testis), while a strong circular signal in the outer layer is typical for females (*Resende* et al. 2014). In some male gonads a blood flow signal on the lateral contour represents the pampiniform plexus. Equine fetal genitalia do not change in form from the fifth month of gestation, though they change in size (*Bucca* 2011). Gender determination in advanced gestation can also be performed by identification of external genitalia (*Holder* 2011; *Bucca* 2011).

Various applications for 3D ultrasound exist in human medicine. It has been used for biopsy and staging of rectal cancer, breast examination and diagnosis of fetal and gynecological anomalies (*Hünerbein* 2003; *Correa* et al. 2006; *Gemmeke & Ruiter* 2007). 3D ultrasound has been recently established in veterinary medicine for pregnancy monitoring in dogs and cats, evaluation of gastric affections in dogs, bladder disease diagnosis and kidney ultrasound (*Hildebrandt* et al. 2009; *Pal* et al. 2015; *Dinesh* et al. 2015; *Dehmiwal* et al. 2016). Tomographic ultrasound imaging (TUI) is the sonographic equivalent of computed tomography scanning. Static or dynamic 3D volume datasets are scanned with a 3D ultrasound device and an infinite number of 2D planes of a volume are acquired. TUI allows division of a 3D volume into an individual number of slices with particular slice sickness. The process of division is similar to 3D computed tomography scanning (CT) and magnetic resonance imaging (MRI) and allows a simultaneous visualization of parallel planes in a single screen. TUI studies in human medicine were performed among others for evaluation of the normal fetal heart, as well as for evaluation of fetal cardiac defects (*Viñals* et al. 2003; *Jeanty* et al. 2007; *Ahmed* 2014). Color Doppler can be used in TUI to visualize blood flow in several slices. Screening exams of TUI images can be performed offline on a workstation using external software programs (*Nelson & Pretorius* 1998).

Fetal gender determination in the equine by experienced veterinarians achieved varying results of accuracy previously published (65%, >90%, 100%), when determining fetal sex by different approaches (*Mari* et al. 2002; *Resende* et al. 2014; *Tönissen* 2016). This study aims to determine accuracy of evaluation of combined sonographic techniques – B-Mode, Doppler-, and 3D TUI mode – to determine fetal sex in mares during mid- to advanced-gestation by transabdominal scanning.

Materials and methods

Animals

Transabdominal pregnancy diagnosis and gender determination was performed in fall 2015 and 2016 on 669 mares on various Thoroughbred stud farms in the mid-west of Germany during the annual late pregnancy check-ups of the German Thoroughbred Owners' and Breeders' Association. All mares were Thoroughbreds and the age of the animals varied between 3 and 24 years (2015: 11.2 ± 4.1 years, 2016: 9.9 ± 4.6 years). Pregnancy was progressed between 85 and 231 days (2015: 160 ± 33 days; 2016 165 ± 31 days) of gestation, which was calculated from breeding records.

Equipment, surroundings and preparation of the mare

Examination gloves, at least 70% alcohol-containing disinfectant, a sponge for moisturizing the ventral abdomen and a nose twitch were used as basic equipment. Examinations of the mares were performed in their box, while they were fixed by an assisting person using a halter. If mares were uncooperative and did not tolerate examination, a nose twitch was used for restrainment. The examiner's right shoulder was positioned close to the left flank and the ultrasound probe was held with the right hand. Examiner's viewing direction was up to the mare's head, allowing the veterinarian to be in a comfortable position for examination. The ultrasound device was held by an assisting person, close enough to be accessible to operate, yet far enough to be out of the mare's range. A dark surrounding was ensured to allow optimal diagnostic conditions. Mares were examined in a routine screening program for the German Thoroughbred Association. Therefore, time for examination and pregnancy checks/gender diagnosis was limited to a maximum of 3 minutes for each mare. Sedation was not necessary in any case.

Examinations were carried out with a portable 3D ultrasound device. A Voluson I® (GE Healthcare®, Wauwatosa, WI) was used in this study. As probes, the RealTime-4D-convex-transducers RAB4-8-RS® or RAB2-8 (GE Healthcare®, Wauwatosa, WI) with 2–8 MHz and a penetration depth of 10–30 cm were used. Alcohol containing disinfectant was applied with a sponge to the ventral abdomen between udder and xiphoid, as well as to the probe. Clipping was not performed because examinations were carried out in late summer or fall, even though clipping may be necessary in winter to allow accurate imaging.

Scanning technique

Scanning started in front of the udder and continued along the ventral abdomen. If fetal parts were identified, orientation of the fetus was established. Profound knowledge of fetal anatomy as well as of fetal anatomy ultrasound presentation were needed.

Criteria for gender determination in advanced gestation

Gender determination in our study was based on fetal gonadal structure, specific structure of blood flow in the gonad and external genitalia. As an additional feature, 3D imaging of the fetal gonad was performed and analyzed with software. Gender diagnoses performed in 2015 and 2016 were compared to the gender at birth in 2016 and 2017 respectively and accuracy of correct gender determination was calculated.

A fetus was diagnosed as male, if the gonads were located close to the bladder and appeared longitudinally oval in shape and homogenously echodense in B-Mode scanning. Sometimes a hyperechogenic line (representing mediastinum testis) along the longitudinal axis could be displayed. Doppler sonography showed intense blood flow along the central line, representing the blood flow in the mediastinum testis. In some male gonads a blood flow signal on the lateral contour represented the pampiniform plexus (figure 2)

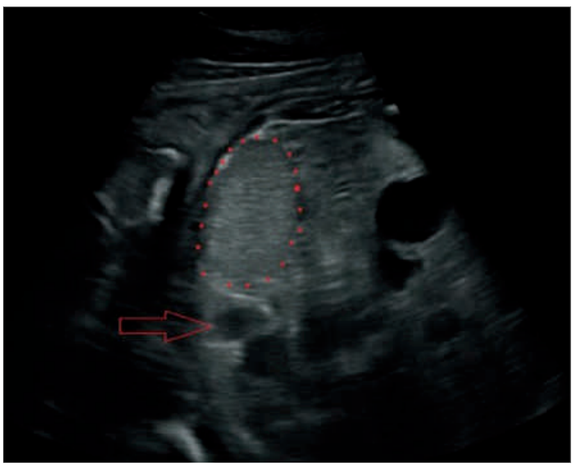

Fig 1 Stored picture of a B-Mode ultrasound video of a mare at 186 days of gestation. The fetal gonad is marked with red dots. The gonad is of homogenous structure and longitudinal-oval form, representing a male fetus. The gonad is positioned close to the bladder (red arrow).
Aufgezeichnetes Bild eines B-Mode Videos einer tragenden Stute am 186. Trächtigkeitstag. Die fetale Gonade ist mit roten Punkten umkreist. Die Gonade zeigt eine homogene Echotextur, eine längsovale Form und stellt damit eine männliche Gonade dar. Die Position der Gonade im fetalen Abdomen befindet sich in unmittelbarer Nähe zur fetalen Harnblase (roter Pfeil).

Fig. 2 Doppler ultrasound image of male gonads at 211 days of gestation of a mare. The Doppler signal shows an intense central blood flow representing the testicular vein. Furthermore, blood flow can be detected on the surface of the gonad.
Doppler Ultraschallbild einer männlichen fetalen Gonade am 211. Trächtigkeitstag. Im Zentrum der Gonade ist ein starkes Durchblutungssignal sichtbar, das einen Blutfluss in der Zentralvene darstellt.

The fetus was diagnosed as female if gonads were located close to the kidneys and if a differentiation between cortex and medulla (echodense core, hyperechogenic cycle) was visible in B-Mode ultrasound. A strong circular Doppler signal was visible in the outer layer representing blood flow in the cortex (figure 4). In some female gonads a strong blood flow signal on the edge of the gonad represented vascularization of the ovarian artery. B-Mode videos of the fetus and Doppler sonography of the gonads were videotaped to allow further analysis. A 3D picture was taken in 3D static mode and vol-

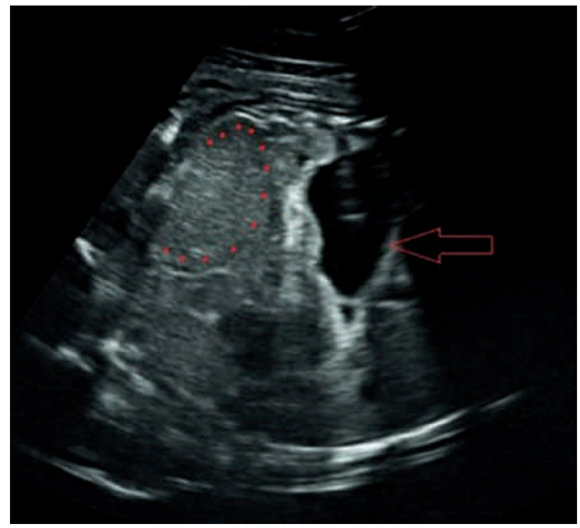

Fig. 3 B-Mode image of a fetus at 183 days of gestation of a mare. The hypoechogenic stomach can be seen in the thoracic captivity (red arrow), fetal gonads are of bizoned echogenicity. Cortex of the gonad is marked with red dots.
B-Mode Bild eines Fetus am 183. Trächtigkeitstag. Der hypoechogene Magen liegt in der Brusthöhle (roter Pfeil). Die fetale Gonade stellt sich zweizonig dar, die Rindenschicht der Gonade ist mit roten Punkten gekennzeichnet.

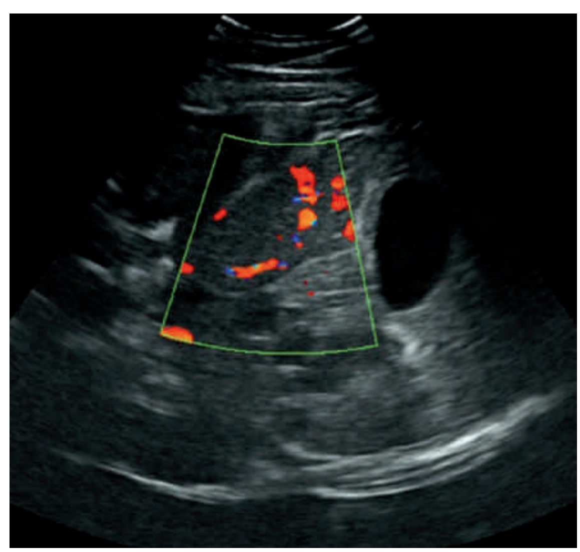

Fig. 4 Doppler ultrasound of a female fetal gonad at 183 days of gestation of a mare. Intense blood flow can be diagnosed in the cortex, whereas no blood flow can be detected in the center of the gonad. Blood flow on the outer surface on the right top represents blood flow in the ovarian artery.
Doppler Ultraschall einer weiblichen Gonade am 183. Trächtigkeitstag. Ein intensiver Blutfluss ist in der Rindenschicht sichtbar, im Zentrum der Gonade (Markzone) kann kein Blutfluss dargestellt werden.

ume data was obtained. Therefore, the fetal gonads were determined as regions of interest and pictures were stored to the internal storage. Obtaining good quality pictures for further analysis was sometimes time consuming and patience of the mare was necessary. The mare had to be stand still for a few seconds to guarantee usable pictures for further analysis. Fetal and mare's movements caused blurred images that were not appropriate for further analysis. Various pictures were stored for each gonad to allow later analysis. Evaluation of the pictures was either made with the on-board software or with the 4D-View® software (GE Medical Systems Kretztechnik, Zipf, Austria) on an external workstation. The 3D image volumes were analyzed using Tomographic Ultrasound Imaging (TUI) (4D View® Version 10.x, GE Healthcare, Austria). The gonads were cut into slices of defined thickness and slices were evaluated due to the anatomical structure.

Results

Transabdominal pregnancy examinations were performed on Thoroughbred mares pregnant from day 85 to 231 (2015: 160 ± 33 days; 2016: 165 ± 31 days).

Transabdominal gender diagnosis was performed on 386 pregnant mares in September 2015. Gender of the fetus could be determined in 297 cases (~77 %) in a three-minute examination time frame (second + third month of gestation: 51 %, forth month of gestation: 71 %, fifth month of gestation: 88 %, sixth month of gestation: 80 %, seventh month of gestation: 67 %). The 3D imaging was possible in 118 cases (~40 %). 2D B-Mode videos and Doppler videos were analyzed according to the criteria stated in the chapter 'Materials and Methods'. The obtained 3D volume images were analyzed with TUI and equine fetal gonads were evaluated for their structure, form and location in the fetal abdominal captivity. Male fetal gonads showed a homogenous echostructure, a longitudinal oval form and sometimes the pampiniform plexus could be visualized in 3D TUI (figure 7+8). A hypoechogenic line representing the testicular vein could be seen centrally in some cases, if the ultrasound beam did hit the longitudinal plane (figure 7).

Female gonads were kidney-shaped in transverse section and the longitudinal section showed a longitudinal-oval form (figure 5 + 6). Echostructure of the female gonads was of bizoned echogenicity, representing cortex and medulla of the fetal gonads. Sometimes the ovarian artery could be visualized (figure 5).

Figures 5–8 show TUI of a female and male gonad. The 3D TUI confirmed gender diagnosis made by 2D B-Mode and Doppler function in 100 cases. 3D TUI imaging allowed a gender diagnosis in 18 cases where B-Mode and Doppler sonography showed no clear results.

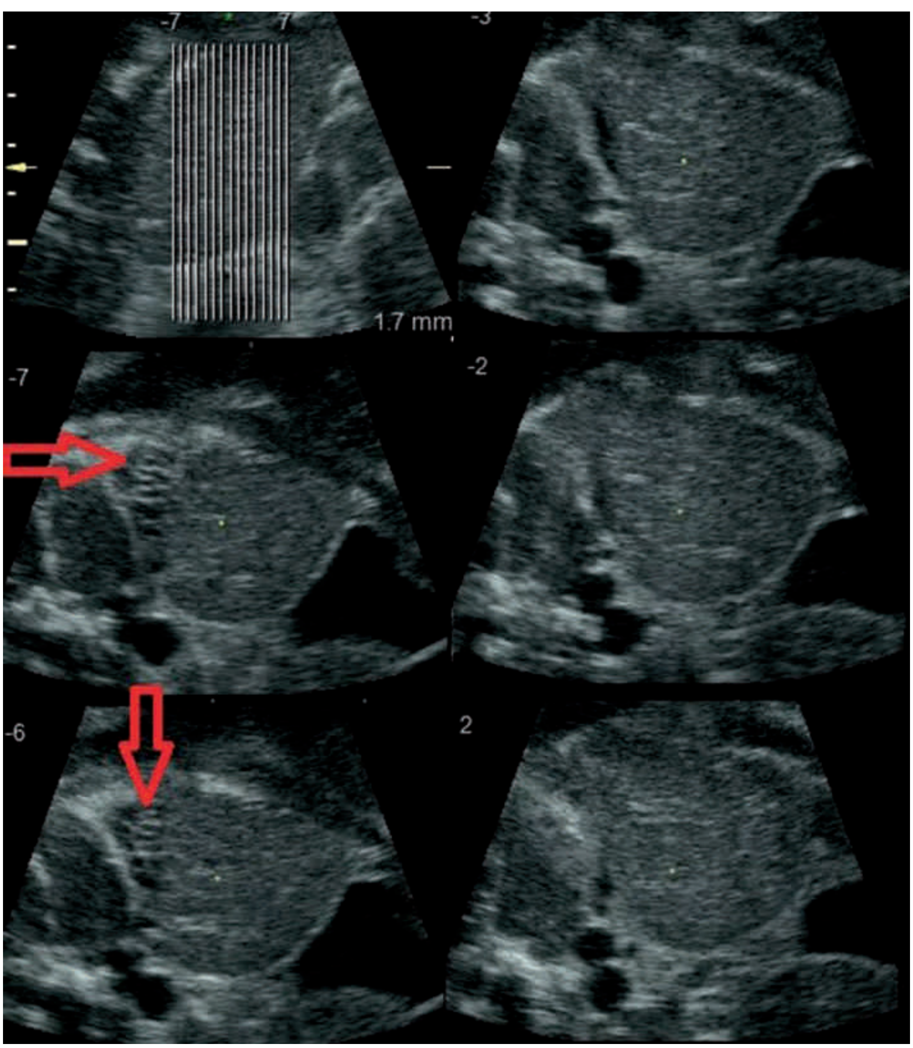

Fig. 5 TUI (tomographic ultrasound imaging) cross section of a female gonad at 183 days of gestation. The 3D volume is cut into 15 slices of 1.7 mm thickness. Process of division is displayed in the sonographic picture in the figure on top on the left. Exemplarily 5 sections are displayed (–7, –6, –3, –2, 2). The ovarian artery can be seen in slices –7 and –6 as hypoechogenic branches (red arrows). The gonad is kidney-shaped and shows bizoned echogenicity.

Tomographische Ultraschallbildgebung (TUI) einer weiblichen Gonade am 183. Trächtigkeitstag. Das 3D Bild wurde in 15 Schnittebenen von 1,7 mm Schichtdicke geteilt. Exemplarisch sind 5 Schnittebenen dargestellt (–7, –6, –3, –2, 2) Die A. ovarica ist in den Schnittebenen –7 und –6 sichtbar (rote Pfeile). Die Gonade ist nierenförmig und zeigt eine zweizonige Echotextur.

Gender predictions were compared to foaling data obtained in summer 2016 to determine the accuracy of gender determination. The postnatal gender of the foal was unknown in 28 cases due to missing feedback of mare's owners or abortion prior to full gestation. This resulted in 269 evaluable cases. The gender of the foal was diagnosed correctly in 254 cases (= ~94%) in 2015 (second + third month of gestation: 93%, forth month of gestation: 96%; fifth month of gestation 93%; sixth month of gestation 97%; seventh month of gestation 100%).

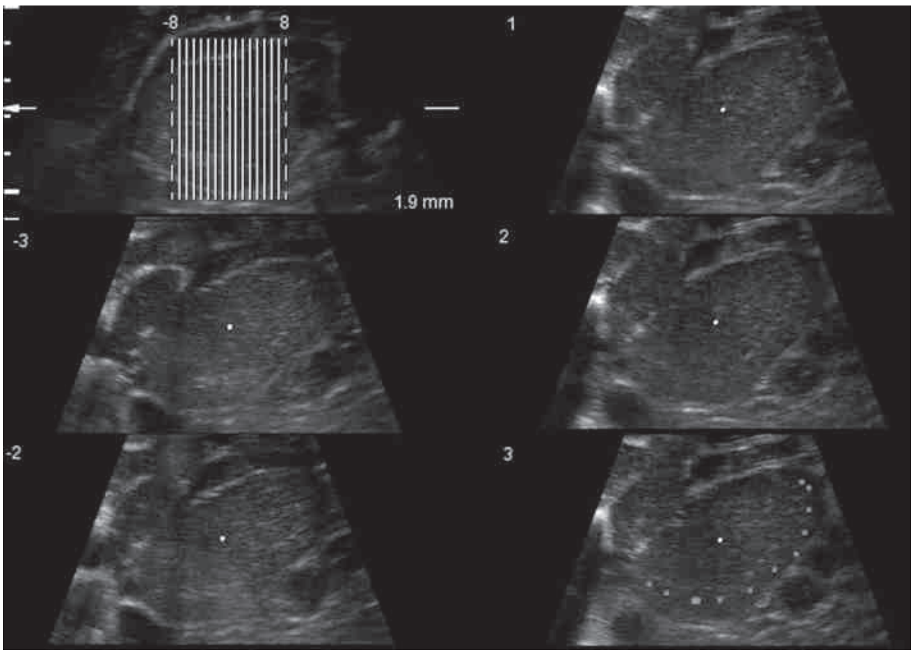

Fig. 6 TUI (tomographic ultrasound imaging) longitudinal section of a female gonad at 163 days of gestation. The 3D volume is cut into 17 slices of 1.9 mm thickness. Process of division is displayed in the sonographic picture in the figure on top on the left. Exemplarily 5 sections are displayed (–3, 2, 1, 2, 3). The gonad shows a bizoned echogenicity, representing cortex and medulla of the gonad. The cortex of the gonad is marked with red dots in slice 3.
Tomographische Ultraschallbildgebung (TUI): Längsschnitt einer weiblichen Gonade am 163. Trächtigkeitstag. Das 3D Bild wurde in 17 Schnittebenen von 1,9 mm Schichtdicke geteilt. Exemplarisch sind 5 Schnittebenen dargestellt (–3, 2, 1, 2, 3). Die Gonade zeigt eine zweizonige Echotextur (Mark- und Rindenzone). Die Rindenschicht der Gonade ist exemplarisch mit roten Pfeilen in Schnittebene 3 markiert

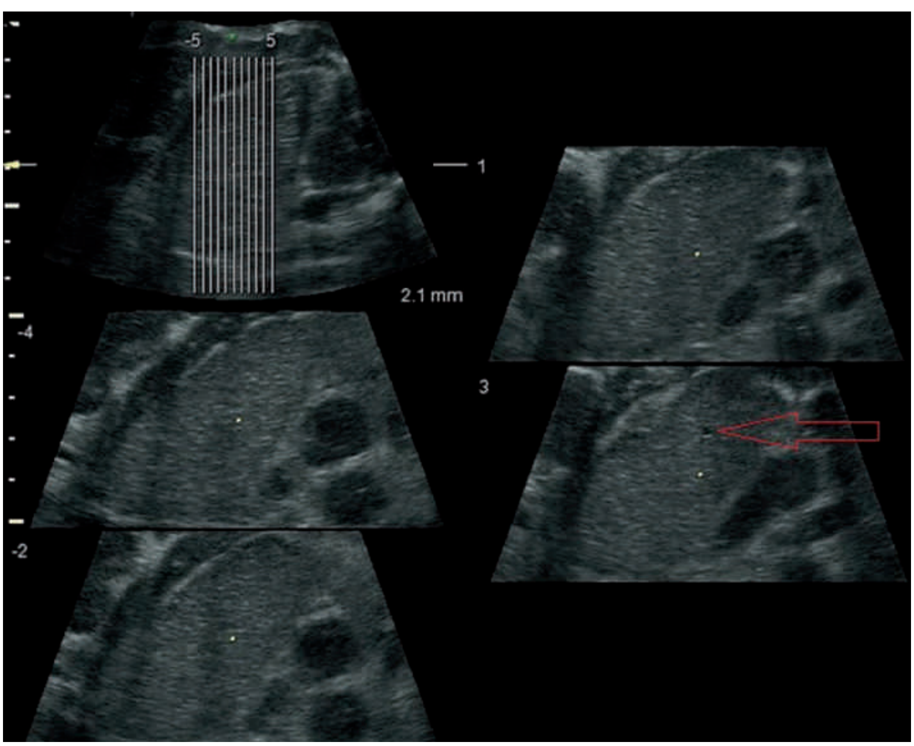

Fig. 7 TUI (tomographic ultrasound imaging) longitudinal section of a male gonad at 181 days of gestation. The gonad is cut into 11 slices of 2,1 mm thickness. Process of division is displayed in the sonographic picture in the figure on top on the left. Exemplarily 4 sections are displayed (–4, –2, 1, 3). The gonad shows a homogenous echostructure and a longitudinal-oval form. A hypoechogenic dot visible in the center of the gonad in slice 3 represents a cross section of the testicular vein (red arrow).
Tomographische Ultraschallbildgebung (TUI): Längsschnitt einer männlichen Gonade am 181. Trächtigkeitstag. Das 3D Bild wurde in 11 Schnittebenen von 2,1 mm Schichtdicke geteilt. Exemplarisch sind 4 Schnittebenen dargestellt (–4, –2, 1, 3). Die Gonade ist von homogener Echotextur. In Schnittebene 3 ist im Zentrum der Gonade ein hypoechogener Bereich sichtbar, der einen Gefäßquerschnitt darstellt (roter Pfeil).

The transabdominal approach was performed on 283 mares in autumn 2016. Gender determination was possible in 184 cases (~65%), no gender could be determined in 99 cases. Gender determination could be performed on highest rates in the fifth month of gestation (84%) followed by sixth (75%) and seventh month (64%). Lowest rates were found in the second and third month of gestation (11%) as well as in the fourth month of gestation (50%). 3D imaging was possible in 94 cases (~51%).

Gender predictions of the examination year 2016 were also compared to foaling data obtained in summer 2017. The postnatal gender of the foal was unknown in 28 cases due to missing feedback of mare's owners or abortion prior to full gestation, which though resulted in 156 evaluable cases. The gender of the foal was diagnosed correctly in 149 cases (=~96%) in 2016 (second + third month of gestation: 66%, forth month of gestation: 96%; fifth month of gestation 98%; sixth month of gestation 95%; seventh month of gestation 100%).

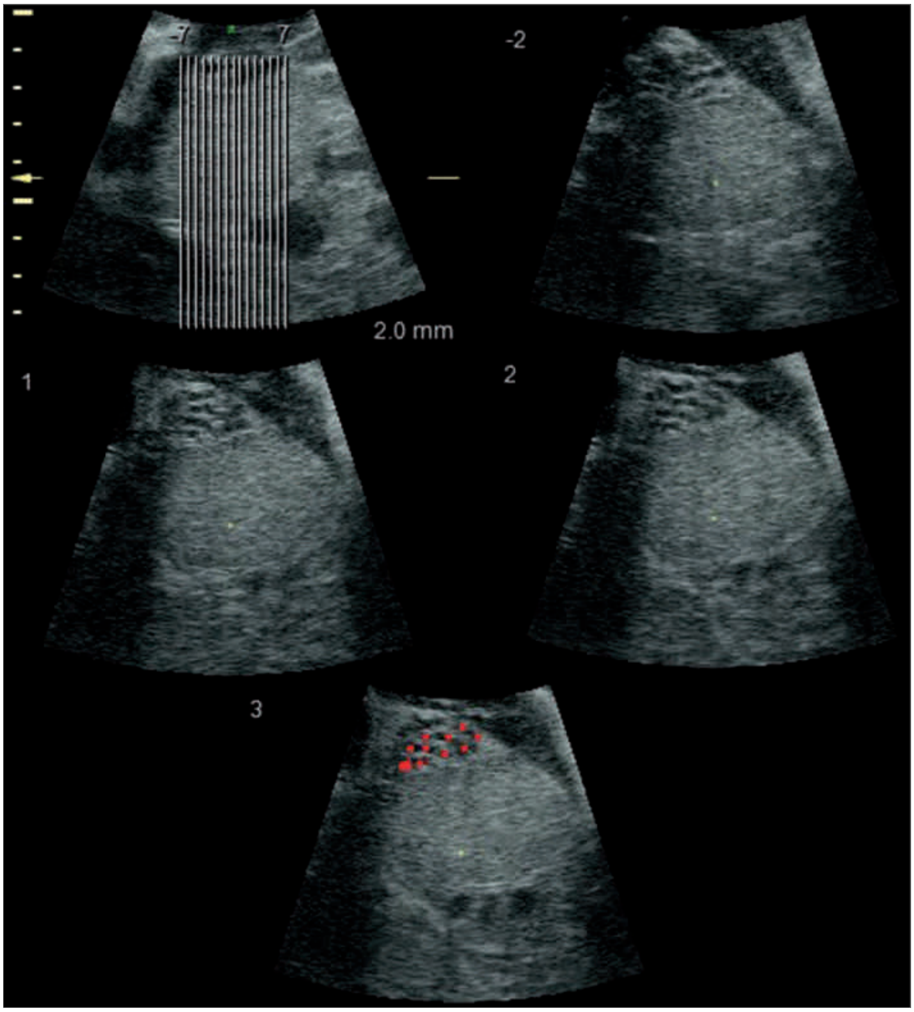

Fig. 8 TUI (tomographic ultrasound imaging) cross section of a male gonad at 193 days of gestation. The 3D volume is cut into 15 slices of 2.0 mm thickness. Exemplarily 4 sections are displayed (–2, 1, 2, 3). The gonad is of homogenous echostructure and of oval shape. The pampiniform plexus can be seen in all slices dorsal to the gonad as hypoechogenic branches. Branches of the pampiniform plexus are marked with red dots in slice 3.
Tomographische Ultraschallbildgebung (TUI): Querschnitt einer männlichen Gonade am 193. Trächtigkeitstag. Das 3D Bild wurde in 15 Schnittebenen von 2,0 mm Schichtdicke geteilt. Exemplarisch sind 4 Schnittebenen dargestellt (–2, 1, 2, 3). Die Gonade ist von homogener Echotextur und zeigt eine ovale Form. Die hypoechogenen Äste des Plexus pampiniformis können in allen Schnittebenen am Dorsalrand der Gonade dargestellt werden. Die Äste des Plexus pampiniformis sind in Schnittebene 3 mit roten Punkten gekennzeichnet.

The results obtained in 2015 and 2016 are shown in Table 1.

Capturing 3D image volumes of the fetal gonads takes a few seconds. Thus repeated recordings of 3D volumes may be required to obtain images that are suitable for further analysis. Movements of either the mare or the fetus may complicate image obtainment. The duration needed for examination decreased with experience of the examiner in handling the 3D ultrasound device, as well as with increasing experience in identifying fetal structures. Reviewing 3D data and performing tomographic ultrasound analysis can take up to 5 minutes for each obtained 3D picture. Reviewing includes identifying the best planes for analysis of the fetal gonad as well as modifying image parameters to obtain high quality images.

Figures 1 and 3 show image sections derived from 2D B-Mode ultrasound videos. Figures 2 and 4 show Doppler ultrasound performed on the equine fetal gonad.

Discussion

Gender determination of the equine fetus has become of bigger interest for the owners of a mare, particularly in regard to breeding purposes. Transabdominal pregnancy diagnosis and gender determination are less invasive than the transrectal approach and can be performed in a substantially bigger time frame (*Bucca* 2011, *Schönbom* et al. 2015, *Tönissen* et al. 2015). The well-being of the fetus can be monitored almost throughout the complete advanced gestation by transabdominal ultrasound. Factors indicating fetal well-being include heart beat rate, movement, aortic diameter and fetal size (*Bucca* et al. 2005). Fetal fluids can be visualized and evaluated for their consistency and echogenicity and the combined thickness of uterus and placenta (CTUP) can be measured (*Reef* et al. 1995). Our study indicates that transabdominal pregnancy diagnosis can be performed as early as around day 80 of gestation and thence can be performed to term. Ultrasound devices and probes that guarantee large penetration depth may be helpful in mid gestation due to fetal positioning in the pelvic captivity. Transabdominal gender determination has recently been the focus of studies for practicability and repeatability (*Tönissen* et al. 2016). Videotapes of transabdominal pregnancy check-ups were shown to various vets,

Tab 1 *Results obtained in 2015 and 2016 | Untersuchungsergebnisse von 2015 und 2016*

	Year of examination	
	2015	2016
Total number of transabdominal pregnancy ultrasound	386	283
Gender diagnosis possible	77%	65%
3D imaging possible	40%	51%
Gender of the fetus diagnosed correctly	94%	96%

and participants were asked to determine fetal gender based on B-Mode and Doppler ultrasound. *Tönissen* et al. (2016) showed that the level of veterinarians' experience in equine reproduction did not correlate with the number of correct gender determinations. They showed that Doppler sonography might facilitate gender determination additionally to B-Mode ultrasound. Nevertheless, this study concluded that the high level of error shown was mainly based on inexperienced raters that were not familiar with the technique of transabdominal ultrasound (*Tönissen* et al. 2016).

Fetal gender determination by experienced veterinarians achieved varying results of accuracy previously published (50%, 65%, >90%, 100%), when determining fetal sex by different approaches (*Mari* et al. 2002; *Resende* et al. 2014; *Tönissen* et al. 2016). Our study shows that a combination of B-Mode ultrasound, Doppler sonography and 3D TUI leads to high accuracies (~92–~96%) of a correct gender diagnosis. The fetal gonads can be scanned in 3D volumes if the fetus is staged in a good position and the mare is stationary. In our studies, 3D volumes and TUI could be performed in ~40% of all conducted gender determinations in 2015 and in ~51% of the examinations in 2016. Determination of the equine gender can be best performed between the 4th–5th month of gestation (*Tönissen* et al. 2015, 2016); nevertheless, repeated examination of younger and older fetuses may also allow an accurate gender determination. Our study emphasizes with the results shown by *Tönissen* et al. (2016). Highest rates of gender determinations were also shown in the fourth to sixth month of gestation in our study (50–88%). Lower rates of gender determination rates were shown in the second–third as well as in the seventh month of gestation (11–67%). Reasons for lower rate in second–third month include the small size and the intra pelvic position of the fetus. Scanning the fetus, positioned high in the abdominal cavity close to the pelvis, is often challenging. Ultrasound scanning of early advanced pregnant mares includes positioning the probe in regions of the knee fold or close to the udder. Mares tend to show defensive movement when being scanned in those regions, complicating suitable image obtainment for further analysis.

Low rates of gender determinations in advanced pregnancy (seventh month) include fetal positioning or acoustical shadows that may impair presentation. Fetal reproductive organs do not change in shape from 150 days to term, though the size changes and visibility of the structures varies. The determination of external genitalia gets more difficult in advanced gestation because of fetal growth and acoustic shadows caused by bones or the umbilical cord. With increasing size of the gonads, their visualization gets easier and characteristic vascularization can be shown in Doppler sonography.

For the 2015 examination period, 3D TUI confirmed gender diagnosis made by 2D B-Mode ultrasound and Doppler sonography in 100 cases. 3D TUI imaging allowed a gender diagnosis in 18 cases in which B-Mode and Doppler measurement showed no clear results. TUI of the gonads helps to analyze internal structures of the gonad to identify anatomical features. Female gonads were easier to identify due to their bizoned echogenicity. One always has to consider that data was obtained in a large screening and time for examination was limited to three minutes. To avoid motion artifacts in generated 3D volumes, because of fetal or maternal movement, repeated recordings of 3D images are required. This is a clear disadvantage in 3D imaging. It includes capturing a perfectly focused fetal structure in a single view to complete the examination in a short interval (*Kotoyori* et al. 2010; *Kotoyori* et al. 2012). Transrectal 3D ultrasound imaging was published as an additional feature for evaluation of fetal structures and well-being. *Kotoyori* and coworkers showed that 3D imaging in the first half of gestation may help to find an accurate gender determination in the equine fetus (*Kotoyori* et al. 2010; *Kotoyori* et al. 2012). Our study indicates that transabdominal 3D imaging of the horse fetus as additional diagnostic feature to 2D B-Mode and Doppler ultrasound can lead to high values of a correct gender determination. The duration needed for examination is always dependent on the experience of the examiner and the resistance each mare might show. Transabdominal fetal gender determination can be performed within minutes if the mare is cooperative and the fetus is appropriately positioned. In a study performed by *Merkt* et al. (1999) time for examination varied between 4.3 ± 1.2 and 5.5 ± 3.0 minutes for the transrectal approach of gender determination between day 50 and 90 of gestation. (*Curran & Ginther* 1991) showed that time needed for accurate gender determination varied between 16 seconds and 3 minutes 55 seconds for transrectal ultrasound identification of the genital tubercle between 50 and 99 days of gestation. *Livini* (2010) showed that average time for gender determination lowers with increasing experience of the examiner. The author showed that with increasing experience the time for examination lowered from around one minute to 30–45 seconds for the transrectal approach (*Livini* 2010). Examinations in our study were limited to a maximum of 3 minutes. Within these 3 minutes, we performed a 2D B-Mode ultrasound to identify fetal structures, Doppler velocity measurements to show blood flow within the gonad and obtained 3D image volumes of the fetal reproductive organs. We did not measure time needed for each mare, but time for examination lowered with increasing experience over the two years. As shown in our study, accurate gender determination can be performed within a short time frame even though additional analysis on external workstations can take additional time.

The large time frame for transabdominal gender determination allows repeated examinations if fetal gonads cannot be displayed on first hand. Storing videos into the internal storage helps to review videos and may help to increase accuracies of gender determination. If gender determination cannot be accomplished because of high activity of the fetus or fetal position, examinations can be postponed due to a large examination window.

Transabdominal examination shows high acceptance in mares with lower stress levels (*Schönbom* et al. 2015). It bears no risk of rectal perforation and sedation is not necessary in most of the cases because mares tend to accept the examination after a couple of minutes. Movement of mare and fetus can be easily compensated and positioning the veterinarian at the left flank guarantees a safe working position. Transabdominal approach allows gender diagnosis even in very small horses where transrectal approach of gender determination is limited because of the size of the animal.

Conclusion

In conclusion, to our knowledge, this is the first transabdominal 3D TUI analysis for equine fetal gender determination. The 3D TUI for transabdominal approach was shown as a useful additional criterion for detailed structural analysis of the fetal gonad during advanced gestation. TUI allows the examiner to evaluate the gonad's inner structure similarly to computed tomography or magnetic resonance imaging. High rates of correct gender determinations can be achieved with an experienced examiner and a combined analysis of B-Mode videos, Doppler sonography and 3D images. 3D TUI may help to allow gender determination even in cases, where B-Mode and Doppler imaging show no clear results. This may furthermore increase correctness of diagnosis. Highest rates of possible gender determinations were shown in the fourth to sixth month of gestation indicating the best time frame for transabdominal ultrasound imaging. We assume that obtaining 3D volumes may be challenging in mares that do not tolerate examination and if fetal activity and movements impair image obtaining. Nevertheless, examinations can be postponed to a later date to perform 3D volume scanning again.

References

Ahmed B. I. (2014) The new 3D/4D based spatio-temporal imaging correlation (STIC) in fetal echocardiography: a promising tool for the future. J. Matern. Fetal Neonatal Med. 27, 1163–1168

Aurich C., Schneider J. (2014). Sex determination in horses – Current status and future perspectives. Anim. Reprod. Sci. 146, 34–41

Bucca S., Fogarty U., Collins A., Small V. (2005) Assessment of feto-placental well-being in the mare from mid-gestation to term: Transrectal and transabdominal ultrasonographic features. Theriogenology 64, 542–557

Bucca S. (2005) Equine fetal gender determination from mid- to advanced-gestation by ultrasound. Theriogenology 64, 568–571

Bucca S. (2011) Fetal gender determination from mid to advanced gestation. In: *McKinnon A. O:, Squires E. L:, Vaala W. E:, Varner D. D.* (eds.), Equine Reproduction 2nd Edition, Ames: Wiley-Blackwell; 2011, 2094–2098

Correa F. F., Lara C., Bellver J., Remohí J., Pellicer A., Serra V. (2006) Examination of the fetal brain by transabdominal three-dimensional ultrasound: potential for routine neurosonographic studies. Ultrasound Obstet. Gynecol. 27, 503–508

Curran S., Ginther O. J. (1991) Ultrasonic determination of fetal gender in horses and cattle under farm conditions. Theriogenology 36, 809–814

Curran S, Ginther O. J. (1989) Ultrasonic diagnosis of equine fetal sex by location of the genital tubercle. J. Equine Vet. Sci. 9, 77–83

Dehmiwal D., Behl S. M., Singh P., Tayal R., Pal M., Chandolia R. K. (2016) Diagnosis of pathological conditions of kidney by two-dimensional and three-dimensional ultrasonographic imaging in dogs. Vet. World 9, 693–698

Dehmiwal D., Behl S. M., Singh P., Tayal R., Pal M., Chandolia R. K. (2015) Diagnosis of urinary bladder diseases in dogs by using two-dimensional and three-dimensional ultrasonography. Vet. World 8, 819–822

Gemmeke H., Ruiter N. V. (2007) 3D ultrasound computer tomography for medical imaging. Nuclear Instruments and Methods in Physics Research A 580, 1057–1065

Hildebrandt T. B., Drews B., Kurz J., Hermes R., Yang S., Göritz F. (2009) Pregnancy Monitoring in Dogs and Cats Using 3D and 4D Ultrasonography. Reprod. Dom. Anim. 44, 125–128

Holder R. D. (2011) Fetal sex determination in the mare between 55 and 150 days of gestation. In: *McKinnon A.O., Sqquires E.L., Vaala W.E., Varner D.D.* (eds), Equine Reproduction 2nd Edition, Ames: Wiley-Blackwell, 2080–2093

Hünerbein M. (2003) Endorectal ultrasound in rectal cancer. Colorectal Disease 5, 402–405

Jeanty P., Chaoui R., Tihonenko I., Grochal F. (2007) A review of findings in fetal cardiac section drawings, part 1: The 4-chamber view. J. Ultrasound Med. 26, 1601–1610

Kotoyori Y., Yokoo N., Ito K., Murase H., Sato F., Korosue K., Nambo Y. (2012) Three-dimensional ultrasound imaging of the equine fetus. Theriogenology 77, 1480–1486

Kotoyori Y., Yokoo N., Ito K., Kimura Y., Korosue K., Ashihara N., Nambo Y. (2010) Transrectal 3-dimensional ultrasound examination of the equine fetus during the first half of gestation. Anim. Reprod. Sci. 121, 327–328

Livini, M. (2010) Determination of fetal gender by transrectal ultrasound-examination: Field`s experience. Proceedings of the Annual Convention of the AAEP 56, 323–327

Mari G., Castagnetti C., Belluzzi S. (2002) Equine fetal sex determination using a single ultrasonic examination under farm conditions. Theriogenology 58, 1237–1243

Merkt H., de Andrade Moura J. C., Jöchle W. (1999) Gender determination in equine fetuses between days 50 and 90 of pregnancy. J. Equine Vet. Sci. 19, 90–94

Nelson T. R., Pretorius D. H. (1998) Three-dimensional ultrasound imaging. Ultrasound Med. Biol. 24, 1243–1270

Pal M., Singh P., Tayal R., Dehmiwal D., Behl S. M., Kumar S., Chandolia R. K. (2015) A comparative study of two-dimensional and three-dimensional ultrasonography in evaluation of gastric affections in dogs. Vet. World 8, 707–712

Reef B., Vaala E., Worth T., Hammett B. (1995) Ultrasonographic evaluation of the fetus and intrauterine environment in healthy mares during late gestation. Vet. Radiol. Ultrasound 36, 533–541

Renaudin C. D., Gillis C. L., Tarantal A. F. (1997) Transabdominal combined with transrectal ultrasonographic determination of equine fetal gender during midgestation. Proceedings of the Annual Convention of the AAEP 43, 251–255

Resende H. L., Carmo M. T., Ramires Neto C., Alvarenga M. A. (2014) Determination of equine fetal sex by Doppler ultrasonography of the gonads. Equine Vet J 46, 756–758

Schönbom H., Kassens A., Hopster-Iversen C., Klewitz J., Piechotta M., Martinsson G., Kißler A., Burger D., Sieme H. (2015) Influence of transrectal and transabdominal ultrasound examination on salivary cortisol, heart rate, and heart rate variability in mares. Theriogenology 83, 749–756

Tönissen A., Martinsson G., Otzen H., Schürmann K., Schütze S., Ertmer F., Kassens A., Sielhorst J., Brehm R., Sieme H. (2015) To perform fetal gender determination in the mare by ultrasound during early and advanced gestation. Pferdeheilkunde 31, 153–158; DOI 10.21836/PEM20150207

Tönissen A., Martinsson G., Pricking S., Otzen H., Ertmer F., Rau J., Sielhorst J., Rohn K., Sieme H. (2016) Transabdominal ultrasonographic determination of fetal gender in the horse during mid-gestation – a comparative study using randomized video images to investigate variation in diagnostic performance among raters, and the effect of month of gestation. Pferdeheilkunde 32, 29–35; DOI 10.21836/PEM20160105

Viñals F., Poblete P., Giuliano A. (2003) Spatio-temporal image correlation (STIC): a new tool for the prenatal screening of congenital heart defects. Ultrasound Obstet. Gynecol. 22, 388–394