Acta Scientiae Veterinariae, 2011. 39(Suppl1): s123 - s134.

ISSN 1679-9216 (Online)

Local Effect of the Conceptus on Uterine Vascular Perfusion and Remodeling during Early Pregnancy in Mares - New Findings by Doppler Ultrasonography

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ABSTRACT

***Background*:** The mammalian reproductive tract is the only organ system in the body where entire tissue layers and structures are in physiologically dynamic and cyclic changes. Angiogenesis is well known to be critical to assure blood supply for tissue growth and remodeling. Ovarian-produced steroids control reproductive tract remodeling, and cyclic rhythmicity of the hypothalamic-ovarian axis.

***Review*:** We have proposed that uterine remodeling during pregnancy is modulated by the conceptus. Special attention was paid to conceptus modulation of the uterine vascular and architectural changes prior to implantation in equids. Our studies using Doppler ultrasonography have described vascular and morphological endometrial changes during early pregnancy in mares. The most important vascular changes observed were: 1) Transient changes in endometrial vascular perfusion accompany the embryonic vesicle as the vesicle changes location during embryo mobility. 2) The continued presence of the vesicle in the same horn for an average of 7 min stimulated an increase in vascularity of the endometrium of the middle segment of the horn during mobile phase. 3) After fixation, endometrial vascularity was progressively higher in the following sequence: horn without the vesicle, horn with the vesicle, and area of endometrium surrounding the fixed vesicle. 4) After fixation, an early vascular indicator of the future position of the embryo proper was discovered by color-Doppler imaging and consisted of a colored spot in the image of the endometrium close to the wall of the embryonic pole. In addition to the observed vascular changes, morphological changes also were observed. They are related to asymmetrical encroachment of the uterine wall, resulting from differential thickening of the upper turgid uterine wall at the mesometrial attachment, in which is normally observed in mares after embryonic vesicle fixation. The thickening of the endometrium was studied during mobile phase and after fixation, and the thickness of the endometrium at the mesometrial aspect of the vesicle divided by the thickness at the antimesometrial aspect was termed the encroachment ratio. The most important morphological changes observed were: 1) Differential dorsal thickening of the endometrium that surrounds the embryonic vesicle began during the later days of the mobility phase. 2) After fixation, the differential dorsal thickening or endometrial encroachment upon the vesicle increased rapidly and was more than four times thicker than ventrally by 3 days after fixation. 3) The increase in vascularity began before the increase in the encroachment ratio in the endometrium at the site of future fixation. 4) The increase in the encroachment ratio between 1 and 3 days after fixation was more rapid than during -4 to 0 days before fixation. 5) Embryonic vesicle dysorientation was associated with a flaccid uterus and defective encroachment of the dorsal endometrium. 6) Asymmetric enlargement of the allantoic sac spontaneously corrected the disorientation of the embryo proper in mares with apparently normal uterine tone and endometrial encroachment, so that orientation of the umbilical cord attachment was at a normal position near 12 o'clock.

***Conclusion*:** Our data provided insight into the architectural and vascular changes in the reproductive tract of equids. These results set the stage for future experiments to understand more completely the role of the conceptus in regulating the uterine environment in favor of its development.

Keywords: doppler ultrasonography, conceptus, mares, uterus, vascular perfusion, remodeling.

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I. EARLY PREGNANCY IN EQUIDS

Equids are the only common eutherian mammals in which real-time images of conceptus (embryo, extraembryonic membranes and fluid) migration, fixation, and orientation and conversion from yolk sac to allantoic sac placentation can be studied sequentially *in vivo* and detectable without disturbance. This research capability results from the availability of transrectal ultrasonography, the large size of the fluid-filled embryonic vesicle (conceptus), and the close proximity of the uterine wall to the rectal wall [17].

The equine embryonic vesicle enters a uterine horn on Day 6 (ovulation = Day 0) [3,35]. When first detected by transrectal ultrasonography on Days 9 or 10, the vesicle is frequently (60% of time) in the uterine body [15,25]. Thereafter, the frequency of entries into the uterine horns increases and a phase of maximum mobility begins, involving all parts of the uterus. Maximum mobility extends over Days 12-14 when the vesicle grows from about 9 to about 15 mm in diameter. The mobility favors physiologic exchange between the relatively small conceptus and large uterus [15]. In this regard, results of confinement of the conceptus to one uterine horn indicate that the conceptus locally stimulates uterine turgidity and edema, as well as contractility [24], and that movement throughout the uterus is required to prevent the bilaterally active uterine luteolytic mechanism [24,26]. Cessation of mobility is called fixation and occurs on Days 15-17 [16]. The site of fixation is at a flexure in the caudal segment of one of the uterine horns without regard to the side of ovulation. It has been postulated that fixation occurs at the flexure because it is a physical impediment to continued mobility of the growing vesicle [14,16].

Orientation of the embryonic vesicle refers to the position of the embryonic disc or embryo proper at the periphery of the vesicle (embryonic pole) relative to the position of the mesometrial attachment. The pattern of orientation (antimesometrial versus mesometrial) is fairly constant within species but differs among species [28,29]. When first detected by ultrasound (Days 19 to 22), the equine embryo proper is in the ventral hemisphere of the embryonic vesicle or opposite to the mesometrial attachment [16]. It is unlikely that orientation occurs before embryo mobility ceases. In this regard, simulated embryonic vesicles rotated or rolled during intrauterine location changes [17]. These observations indicate that orientation occurs between the day of fixation (Day 16) and the earliest reported day of ultrasonic identification of the embryo proper (Day 19). It has been postulated [16,17] that equine embryo orientation results from the interaction of at least three factors: 1) differences in tensile strength between the thin (two cell layers) and thick (three layers) portions of the vesicle wall; 2) asymmetrical encroachment of the uterine wall on the vesicle, resulting from differential thickening of the upper turgid uterine wall at the mesometrial attachment; and 3) the massaging action of uterine contractions. A distinct, smooth, and strong capsule encloses the embryonic vesicle until about Day 21 [4] and is an additional factor that likely favors the orientation process. The surface of the equine embryonic vesicle develops adhesive qualities [8] which may aid in anchoring the vesicle after orientation is completed. Disproportional thickening of the dorsal uterine wall occurs by Day 17 and accounts for the nonspherical shapes of the vesicle as it begins to encroach upon the two-layered membrane [16].

The beginning of the implantation process in mares starts around Day 40 of pregnancy but the beginning of the functional placenta is not observed until Day 60, with complete formation of micro-cotyledons about Day 120 [1,32]. Based on the number of tissues separating maternal from fetal blood, the equine placenta is classified as epitheliochorial as all six tissue layers (epithelium, stroma, and endothelium) are present in both maternal and fetal sides [2].

After entering the uterus, the embryo must be detected by the mother and the luteolytic mechanism abrogated so as to maintain progesterone synthesis by the corpus luteum [31]. This is the first luteal response to pregnancy, better known as maternal recognition of pregnancy. High embryo loss rates are common at this time in all domestic animals and women, and are higher if the conception resulted from assisted reproductive techniques [9,27,37]. The mobility phase of the equine embryonic vesicle is well established as an important event to maintain luteal function [26]. The early pregnancy is a critical time for embryo survival in mares, likely to in others mammals species. Many are the factors involved in early embryo loss and, securely, the uterine vascular and architectural changes will support an adequate uterine environment for embryo survival and development. High rate of embryo are reported during the two first months of pregnancy ranging from 2.6% to 24.0% [7,37,39].

II. DOPPLER ULTRASONOGRAPHY

Transrectal B-mode (gray scale) ultrasonography revolutionized diagnosing and monitoring of biologic and pathologic reproductive events in cattle and horses. An important advantage of this technique is that a structure can be evaluated in real time while the area is being scanned systematically. B-mode is used not only to identify and measure structures, but also to assess physiologic status. Doppler ultrasound adds blood-flow information to the B-mode image about anatomy and function [22].

Doppler ultrasound involves two modalities (spectral mode and color-flow mode) with distinctly different methods for targeting an area of interest. For the spectral mode, the blood-flow characteristics in a focused area of a vessel are assessed by placement of a sample gate cursor into the B-mode or color-mode image of the lumen of a targeted vessel. Color-flow imaging estimates blood velocities and encodes and displays it as colored regions superimposed on the B-mode image. The extent of local perfusion or blood flow area within the tissues can be estimated with color flow and quantified directly at the level of the tissue [19].

III. BLOOD FLOW ASSESSED BY DOPPLER ULTRASONOGRAPHY DURING PREGNANCY

Uterine blood flow changes during pregnancy have been an object of interest of many studies. Using electromagnetic probes, it was shown that blood flow increased in the uterine artery ipsilateral but not contralateral to the conceptus between Days 13 and 15 in sheep [30] and Days 15 and 17 in cattle [11]. Swine have embryos in both horns and blood flow transiently increases in both uterine arteries 12 and 13 days after insemination, but when embryos are experimentally confined to one horn, the blood flow increases only on that side [12]; furthermore, blood flow to uterine segments containing a conceptus is greater than for segments that do not contain a conceptus [13]. Blood flow to the pregnant uterus has been shown to be increased in the uterine artery ipsilateral to the embryo proper on Days 14-18 and after Day 25 in heifers [11]. A brief review of the earliest studies on uterine blood flow changes is provided by [10].

Transrectal Doppler ultrasonography was used for noninvasive study of the blood flow in the uterine arteries during early pregnancy in mares [5,6]. Time averaged maximum velocity (TAMV) was higher and resistance index (RI) was lower in the arteries of pregnant mares than in nonpregnant mares beginning on Day 11. From Days 15 to 29 of pregnancy, TAMV was higher and RI lower in the uterine artery ipsilateral to the conceptus than in the opposite artery. The authors indicated that an increase in TAMV represented greater blood flow in the arteries, and a decrease in RI represented reduced resistance to blood flow in the vasculature distal to site of assessment. It was not determined whether conceptus fixation had occurred in at least some mares by the day of detection of a difference in blood flow between the ipsilateral and contralateral arteries. Thus, a local effect of the embryonic vesicle on the uterine vasculature in association with mobility of the conceptus was not demonstrated. Data on uterine vascular changes during early pregnancy in mares assessed by color-mode Doppler ultrasonography have been published [21,33,34] and will be detailed in the next topics of this review. The effect of the conceptus mediating uterine perfusion changes during mobility phase and after embryonic vesicle fixation was studied. In addition, the effects of uterine vascular changes on the endometrial ultrasonographic morphology were also investigated.

IV. LOCAL EFFECT OF THE CONCEPTUS ON UTERINE VASCULAR PERFUSION AND REMODELING DURING EARLY PREGNANCY

4.1 Mobility phase and Fixation.

In our first study [33], color Doppler ultrasonography was used to study the relationships of endometrial vascular perfusion and uterine blood flow to the mobility of the embryonic vesicle during early pregnancy in mares. The equine embryonic vesicle is mobile on Days 12-14 (Day 0 = ovulation), when it is about 9-15 mm in diameter. Movement from one uterine horn to another occurs on average about 0.5 times per hour. Mobility ceases (fixation) on Days 15-17. Transrectal color Doppler ultrasonography was used to study the relationship of embryo mobility (experiment 1) and fixation (experiment 2) to endometrial vascular perfusion (Figure 1). In experiment 1, mares were bred and examined daily from Days 1-16 and were assigned, retrospectively, to a group in which an embryo was detected (pregnant mares; n=16) or not detected (n=8) by Day 12. Endometrial vascularity (scored 1-4, none to maximal) did not differ on Days 1-8 between groups or between the side with and without the corpus luteum. Endometrial vascularity scores were higher ($P < 0.05$) on Days 12-166 in both horns of pregnant mares than in mares with no embryo. In pregnant mares, the scores increased ($P < 0.05$) between Days 10-12 in the horn with the embryo and were higher ($P < 0.05$) than in the opposite horn on Days 12–15 (Figure 2). In experiment 2, 14 pregnant mares were examined from Day 13 to 6 d after fixation (Figure 3). Endometrial vascularity scores and number of colored pixels per cross section of endometrium were greater ($P < 0.05$) in the endometrium surrounding the fixed vesicle than in the middle portion of the horn of fixation. Results supported the hypothesis that transient changes in endometrial vascular perfusion accompany the embryonic vesicle as the vesicle changes locations during embryo mobility.

4.2 Fixation and Orientation

Orientation of the embryonic vesicle refers to the position of the embryo proper at the periphery of the vesicle relative to the position of the mesometrial attachment. In mares, the embryonic pole of the vesicle is antimesometrial after completion of orientation. Day of vesicle fixation, differential thickening of the endometrium near the mesometrial attachment, and orientation of the embryonic vesicle were studied in 30 ponies in our second study presented in this review [34], using B-mode and color-Doppler transrectal ultrasonography (Figure 4). The thickness of the endometrium at the mesometrial aspect of the vesicle divided by the thickness at the antimesometrial aspect was termed the encroachment ratio (Figure 4). An early vascular indicator of the future position of the embryo proper was discovered by color-Doppler imaging and consisted of a colored spot in the image of the endometrium close to the wall of the embryonic pole (Figure 5). The early indicator was detected in each mare 0.5 ± 0.1 days after fixation and 2.5 ± 0.2 days before first detection of the embryo proper. The position of the early indicator when first detected at the periphery of the embryonic vesicle was not different significantly from the position of the embryo proper when first detected. At the future site of fixation, the first increase ($P < 0.05$) in the encroachment ratio occurred between 4 and 1 day before fixation (Figure 6). Results supported the hypothesis that differential thickening of the endometrium precedes orientation and indicated that orientation occurs immediately after fixation.

4.3 Embryonic Vesicle Dysorientation

The phenomenon of dysorientation of the embryonic vesicle is the subject of our third study [21]. Orientation of the embryo proper at the periphery of the equine embryonic vesicle is normally antimesometrial or on the ventral aspect of the embryonic vesicle (6 o'clock relative to 12 o'clock at the center of the mesometrial attachment). An early ultrasonographically detectable vascular endometrial indicator of the future position of the embryo proper has been reported previously and was first detected in a mean 2.5 days before detection of the embryo proper. In the present study, four occurrences of dysorientation of the embryo proper were found in a group of 30 mares (incidence, 13%; Figure 7). When first detected, the early indicator of the clock-face position of the embryonic pole for the dysorientation and normal orientation group, respectively, was 1.3

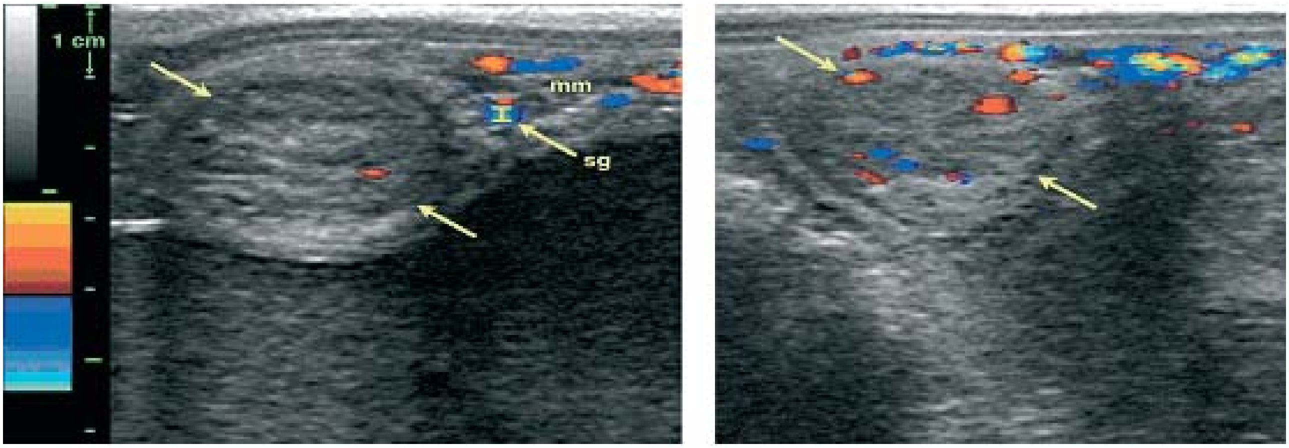

Figure 1. Two images of cross-sections of uterine horns showing minimal (left panel) and maximal (right panel) colored areas of the endometrium from the Doppler flow mode. The sample gate (sg, left panel) indicates the area sampled in the mesometrial attachment (mm=mesometrium) for generating the spectrum used by the scanner in calculating time averaged maximum velocity and pulsatility index. The arrows for each panel delineate the endometrium (reproduced with permission [33]).

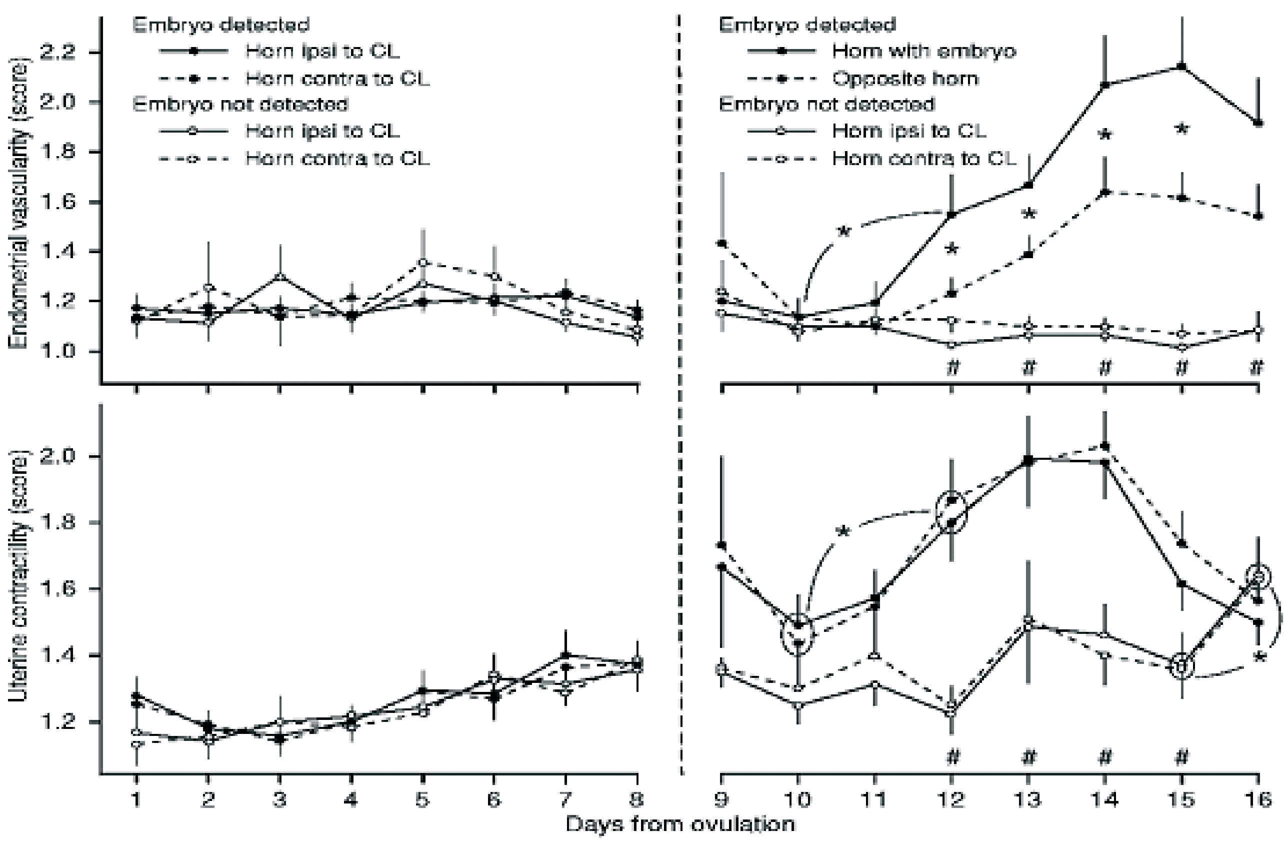

Figure 2. Means ± SEM scores for endometrial vascularity and uterine contractility in bred mares in which an embryo was either detected or not detected by Days 9–12. Number of mares was 16 and 8 for the mares with and without an embryo, respectively, except that only 3, 11, and 15 mares with a detected embryo were available on Days 9, 10, and 11, respectively. The day effect was significant ($P < 0.0001$) for contractility on Days 1–8, and the day-by-group interaction on Days 9–16 was significant for contractility ($P < 0.02$) and vascularity ($P < 0.0001$). An asterisk (*) indicates a day of a difference ($P < 0.05$) between horns within the pregnant group and between days within a group. The pound mark (#) indicates the days of a difference ($P < 0.05$) between pregnant and nonpregnant groups. CL = corpus luteum. Contra = contralateral. Ipsi = ipsilateral. Experiment 1 (reproduced with permission [33]).

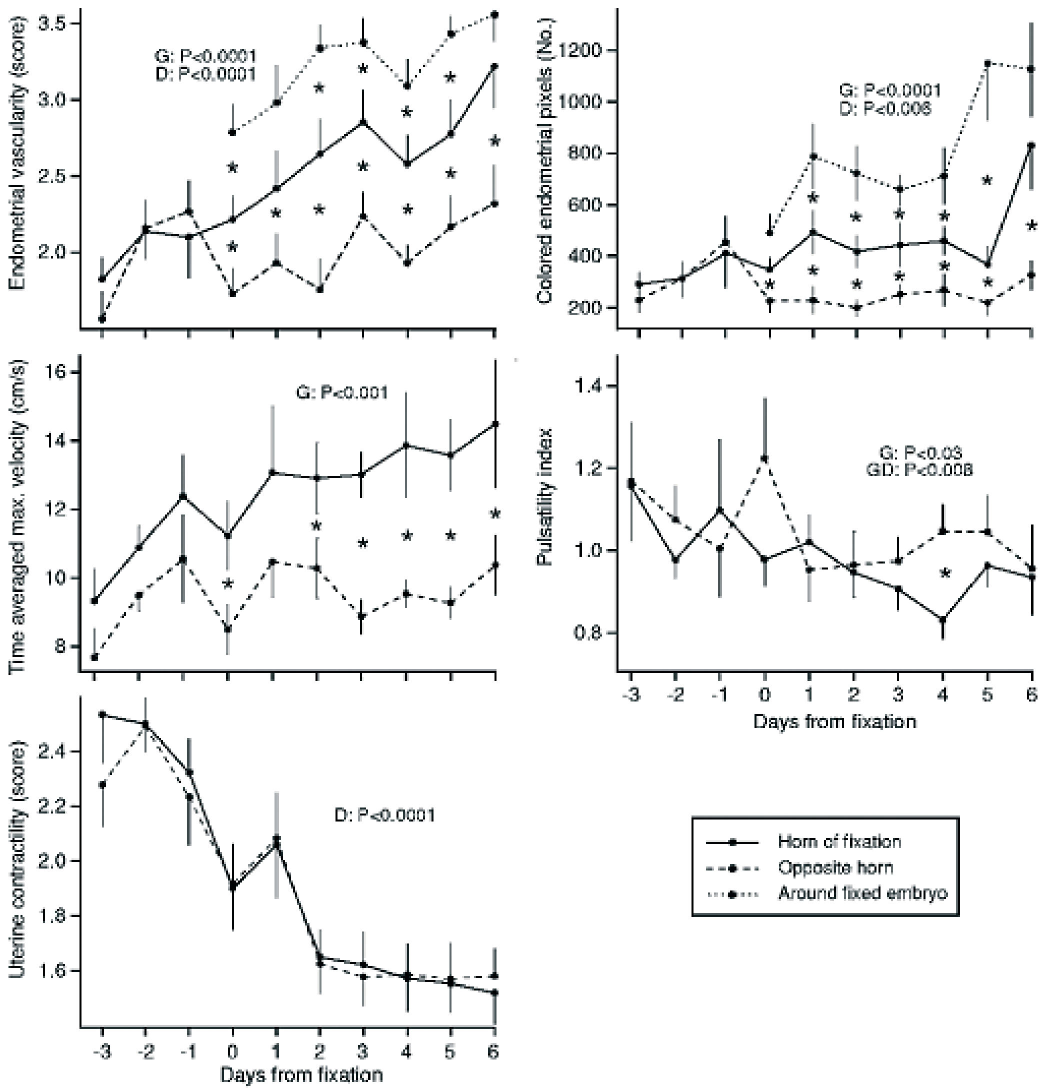

Figure 3. Means ± SEM for uterine color Doppler end points and contractility in the middle segment of the uterine horn of fixation and the opposite horn. The upper two panels include data for the area of endometrium at the location of the fixed vesicle. The scores for vascularity and contractility were from 1–4 for none, minimal, intermediate, and maximal. Significant main effects (G, group; D, day) and the interaction (GD) are shown for the days of and after fixation. An asterisk (*) indicates a day with a difference ($P < 0.05$) between the group above and the group below the asterisk within a day. Experiment 2 (reproduced with permission [33]).

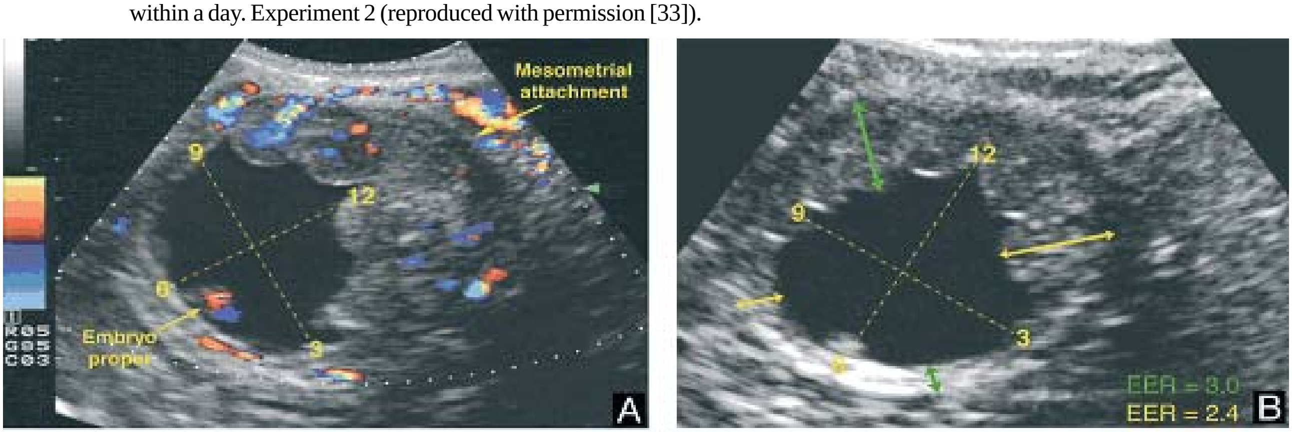

Figure 4. Color mode sonogram depicting the o'clock method of determining the position of a structure at the periphery of the embryonic vesicle (A) and B-mode sonogram illustrating the method for determining the endometrial encroachment ratio (EER; B). The numbers (3, 6, 9, 12) refer to o'clock positions. Alignment with the area of mesometrial attachment is at 12:00 o'clock or 12 h. Endometrial thickness (B) at 10:30 was divided by the thickness at 4:30 (green arrows) and the thickness at 1:30 was divided by the thickness at 7:30 (yellow arrows). The average endometrial encroachment ratio in this illustration is 2.7 (reproduced with permission [34]).

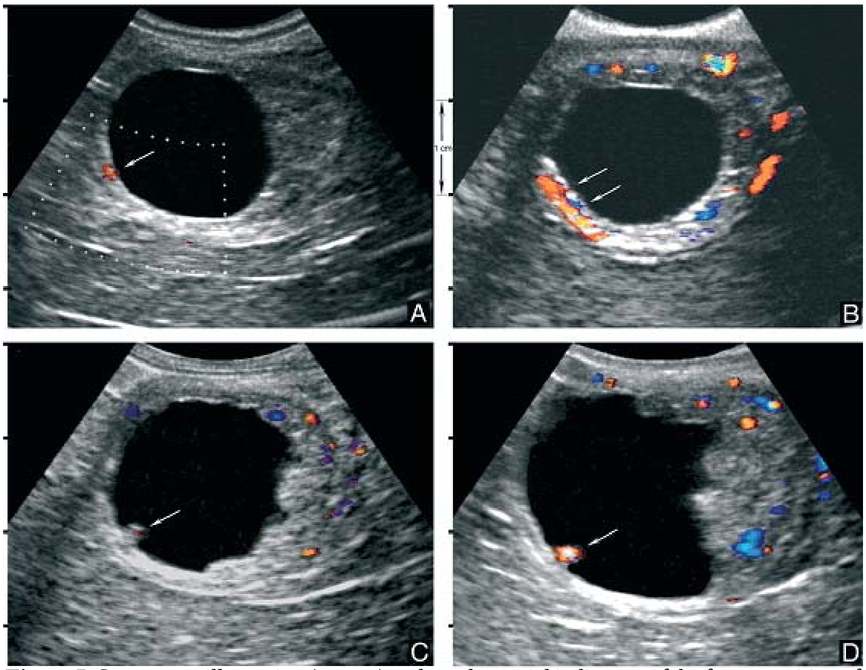

Figure 5. Sonograms illustrating (arrows) early endometrial indicators of the future position of the embryo proper (A, B) and the embryo proper (C, D). The arrows (A, B) indicate sites where the color of the early indicator appears to permeate the vesicle wall, a small embryo proper (note the small color spot; C), and a more developed embryo proper (D). The color-Doppler assessment was confined to the area delineated by the dotted lines (A) to improve the color flow resolution (reproduced with permission [34]).

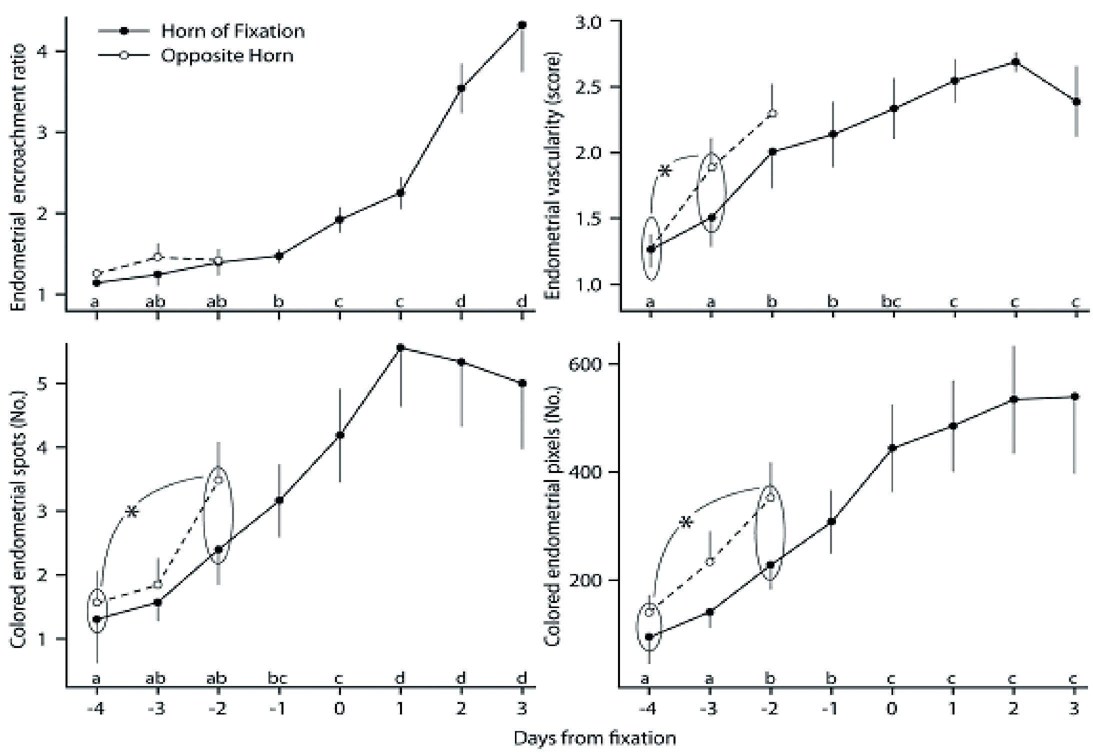

Figure 6. Means (±SEM) for endometrial encroachment ratio (dorsal thickness divided by ventral thickness) and three end points for assessing the extent of vascular perfusion of the endometrium centralized to the day of fixation (n=9 mares). Horn of fixation before Day 0 refers to the future horn of fixation as determined retroactively. The opposite horn refers to the horn in which fixation did not occur. An asterisk indicates a significant difference ($P < 0.05$) between days combined for the two horns. For each panel, lower-case letters above the day axis indicate days of differences between means within the horn of fixation; any two days without a common lower-case letter are different ($P < 0.05$; reproduced with permission [34]).

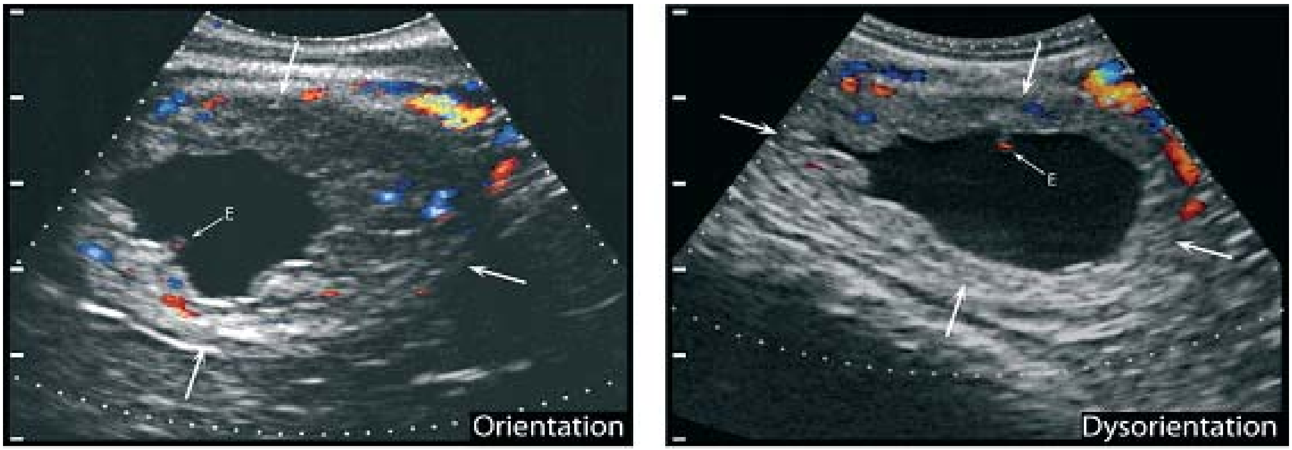

Figure 7. Color-Doppler ultrasonograms of the conceptus at Day 19 for normal orientation and dysorientation. Encroachment or thickening of the endometrium between the mesometrial attachment (upper right) and the embryonic vesicle is prominent for normal orientation but is not apparent for dysorientation. Arrows indicate the periphery of the endometrium. E = embryo proper (reproduced with permission [21]).

± 0.3 and 0.4 ± 0.1 hours from the 6 o'clock position ($P < 0.008$). The extent of dysorientation increased progressively over Days 16 to 19, so that the embryo proper was at 3, 9, 9, or 10 o'clock (Figure 8). Dysorientation was associated with a flaccid uterus and defective encroachment of the dorsal endometrium upon the vesicle in three of the four mares. In a second study, dysorientation of the embryo proper occurred in two mares with apparently normal uterine tone and endometrial encroachment. When first detected, the embryo proper was at 9 or 10 o'clock. However, asymmetric enlargement of the allantoic sac spontaneously corrected the dysorientation, so that orientation of the umbilical-cord attachment was at a normal position near 12 o'clock (Figure 9), at the area of the mesometrial attachment.

V. GENERAL DISCUSSION

Our studies were the first in the literature to use the color-mode of Doppler ultrasonography to assess blood flow in the uterus of mares. In spite of the subjectivity of the color-Doppler evaluation during scored system evaluations, we have demonstrated in all of our experiments with the use of objective validation methods, that this technique is reliable and precise in detecting small variations in blood flow.

The early indicator which consists of a colored spot in the endometrium close to the wall of the embryonic pole and its detection is a good example of the sensitivity of the technique. Based on the early indicator, it was possible to map the position of the embryonic disc from the day of fixation of the embryonic vesicle until visualization of the embryo proper. The embryonic disc is a very active developmental area and the vascular system is the first organ system to be formed during embryogenesis. More detailed

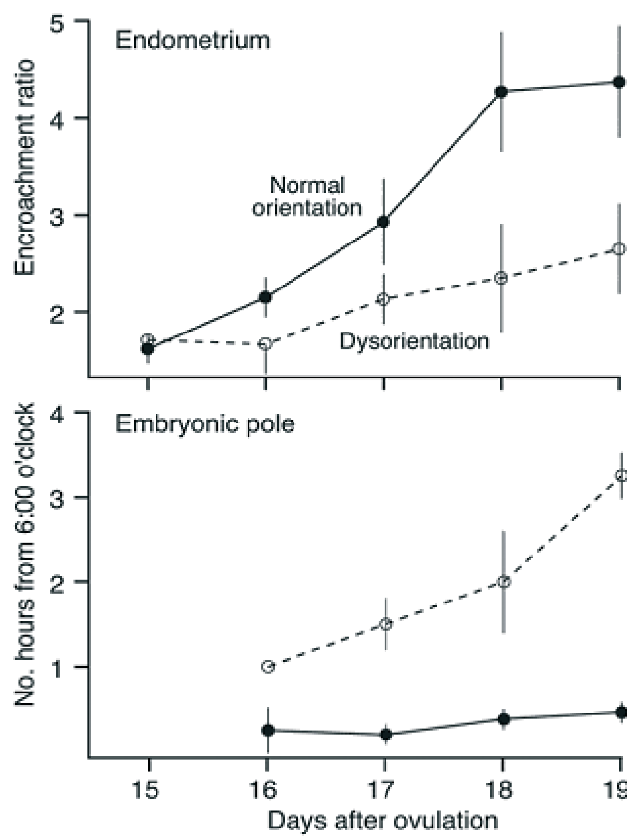

Figure 8. Mean (± SEM) for encroachment ratio and clock-face position of the embryonic pole (number of hours from 6 o'clock). The interaction between group (normal orientation and dysorientation) and day was significant for both end points. Difference between groups was significant ($P < 0.05$) for Days 18 and 19 for endometrium and for each day for embryonic pole (reproduced with permission [21]).

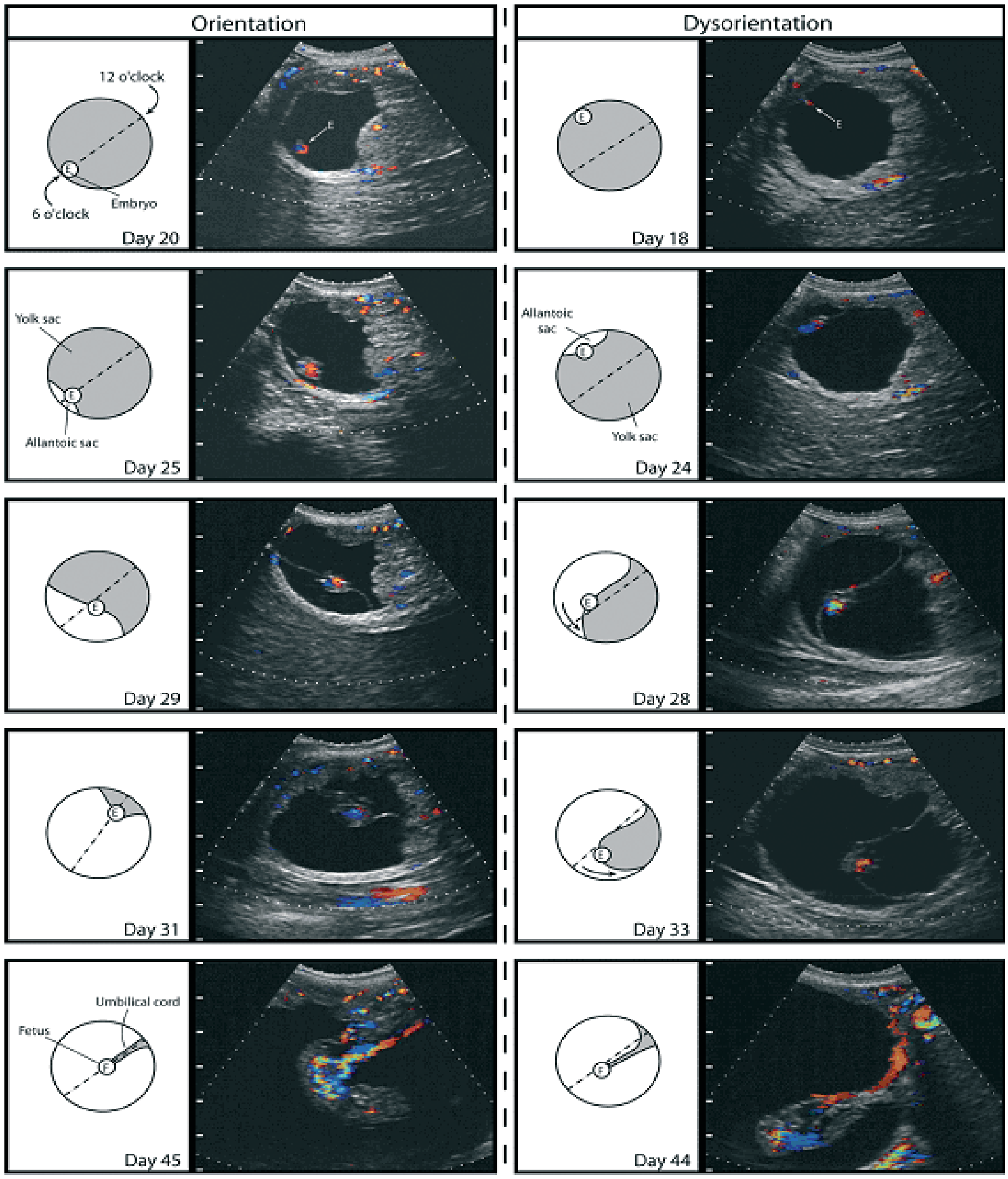

Figure 9. Comparison of the expansion of the allantoic sac for a conceptus with normal orientation and dysorientation of the embryo proper (E). Correction of the dysorientation of the embryo proper occurred by asymmetric expansion of the allantoic sac, so that the orientation of the umbilical-cord attachment of the fetus (F) was in a normal position in the mesometrial area. In the correction of dysorientation of the embryo proper, one end of the opposing membranes of the yolk and allantoic sacs was first to reach the mesometrial area and apparently became adhered. The other unadhered end continued to move toward 12 o'clock as the allantoic sac continued to expand (reproduced with permission [21]).

studies should be done to identify the exact origin of this early indicator or Doppler signals observed in our study. It could reflect endometrial blood flow stimulated by embryonic factors which stimulate vessels in this area of intimate contact through a paracrine action. Another possibility is that the early indicator is formed by contractions of the cardiac muscle cells at the embryonic disc area even before their organization as a heart. Doppler ultrasonography detects signals produced by structures in movement. Contraction of the embryonic cardiac cells interacting with tissues in the immediate area may be sufficiently strong to create tissue movement producing the observed Doppler echoes.

In mares, transient changes in endometrial vascularity accompanied conceptus location changes during the mobility phase and continued presence of the conceptus in the same horn (7-min average) stimulated an increase in vascularity. After fixation, endometrial vascularity was higher in the endometrium surrounding the fixed conceptus, than in other areas of the ipsilateral horn, or in the opposite horn. Differential dorsal thickening of the endometrium preceded embryonic orientation. Based on our studies assessing uterine blood flow during early pregnancy of mares and cows with color Doppler and in the previous studies which other authors used others techniques to assess uterine blood flow in cows, sows and ewes, Equids exhibited the most precocious increase in uterine perfusion during early pregnancy, when compared with bovids [35]. Uterine blood flow changes started to be detected on Day 12 of pregnancy. Two distinct mechanisms could be collaborating to stimulate the increase in uterine vascular perfusion during early pregnancy in mares and these two mechanisms are probably related to two distinct phases of early pregnancy in Equids; the mobility phase and post-fixation phase of the embryonic vesicle. We suggest that the embryonic vesicle could stimulate vasodilation and angiogenesis in the endometrium. These two processes are probably combined. However, during mobility phase, transient rapid changes in uterine blood flow were shown to be related to location of the embryonic vesicle in the uterus. Slight encroachment of the dorsal endometrial was detected during the final days of mobility phase. These results suggest that the rapid transient changes in uterine blood flow reflect vasodilation of blood vessels in the endometrium. More research is necessary to determine which factors provoke this kind of stimulation. The vesicle produce large amounts of estrogens and prostaglandins at this time and these hormones could be involved in vasodilatory mechanisms. In addition, physicochemical interaction of the embryonic vesicle capsule with the endometrial luminal epithelium during conceptus movement may be also considered as a possible factor of stimulation. Angiogenesis is also likely present toward the end of the mobility phase based on the initial dorsal endometrial encroachment and slight increase in endometrial perfusion observed at this time. This idea is further supported by results of the morphometric study [36] which demonstrated increased growth of blood vessels, as well as increased angiogenic factors, in the endometrium adjacent to the fixated conceptus. After fixation, the increase in blood flow and encroachment of the dorsal endometrium is dramatic. Presumably, most of the vascular stimulant factors produced by the embryonic vesicle are highly localized at the site of fixation. Our findings suggest that, while presumptive vasodilation occurs during the mobility phase, the predominant angiogenic stimulus occurs post-fixation.

Orientation of the embryonic vesicle occurred immediately after fixation. Embryonic dysorientation was associated with a flaccid uterus and defective encroachment of the dorsal endometrium. Asymmetric enlargement of the allantoic sac spontaneously corrected dysorientation. Adherence points were found between the yolk sac surface and the dorsal endometrium [36]. These are new findings showing step by step the dynamic interactions between the embryonic vesicle and the uterine wall for the express purpose of aligning the future site of umbilical cord formation to the richest vascular area, the dorsal endometrium and mesometrium. Orientation of the embryonic disc in the ventral endometrial area permits direct contact of the bilaminar layer of the yolk sac at the abembryonic pole with the richest glandular and vascularized area of the endometrium. This positioning favors embryo-maternal exchange guaranteeing survival and development of the early conceptus. It is interesting to observe in Equids that the embryo proper forms ventrally but during development of the allantois is translocated to the dorsal area, the richest endometrial area. At this position, the umbilical cord develops. We have observed that abnormal orientation normally terminates the pregnancy but, in two specific cases when the uterus presented slight tone, the embryonic vesicles were able to correct their orientation by continuing to expand the yolk sac-allantois boundary until it impinged on the dorsal endometrium, permitting formation of the umbilical cord in the correct position. Adherence points on the surface of the conceptus, specifically the bilaminar layer of the yolk sac, are important to maintain conceptus orientation. Studies of the composition of the equine capsule have demonstrated carbohydrate changes during early pregnancy. However, visualization of the adherence points on the yolk sac surface offers a more specific area with which to study biochemical interactions between the surface of the capsule and the endometrial luminal epithelium.

Modulation of the uterine vascular system by the conceptus includes two very distinct and balanced events. The first event consists of stimulation of the uterine architectural and vascular system changes. From the earliest stages of pregnancy to its culmination, the conceptus presumably releases factors driving tissue changes in the uterus, including vascular remodeling, to favor survival and development. However, equally important is the need to limit these uterine changes to prevent overdevelopment. Such may be the case with large offspring syndrome, for example, in which abnormalities in placental vascularization are observed. Many questions are still unanswered, such as whether tissue and vascular remodeling during pregnancy is self-limited or whether this process is mediated by conceptus-produced factors. The processes involved in tissue remodeling of the pregnant uterus and in abnormal conditions of tissue growth, as cancer are similar in appearance. However, during pregnancy tissue growth is limited or controlled in contrast to pathological conditions such as cancer. These thoughts set the stage for a more complete future of investigation. Knowledge of the mechanisms which regulate angiogenic and tissue remodeling of pregnancy would represent an enormous advance in science. Understanding the mechanisms regulating angiogenesis and tissue remodeling in the pregnant reproductive tract will help in the development of therapies for pathological conditions which exhibit abnormal tissue growth.

In summary, our results set the stage for future experiments to understand more completely: 1) the role of the conceptus in regulating the uterine environment to favor its development, including understanding the balance in stimulating and limiting uterine architectural and vascular changes, 2) the role of vascular changes in the regulation of physiological processes in the reproductive tract during cyclicity and pregnancy, and 3) the role of blood flow changes as a practical diagnostic measurer of normal organ and tissue functionality.

REFERENCES

1 **Allen W.R. & Stewart F. 2001.** Equine placentation. *Reproduction, Fertility and Development.* 13: 623-634.

2 **Amoroso E.C. 1952.** Placentation. In: *Marshall's Physiology of Reproduction.* Volume 2. 3rd edn. Parkes A.S. (Ed). London: Logmans Green and Co., pp.127-311.

3 **Battut I., Colchen S., Fieni F., Tainturier D. & Bruyas J.F. 1997.** Success rates when attempting to nonsurgically collect equine embryos at 144, 156 or 168 hours after ovulation. *Equine Veterinary Journal.* 25(Suppl 1): 60-62.

4 **Betteridge K.J., Eaglesome M.D., Mitchell D., Flood P.F. & Beriault R. 1982.** Development of horse embryos up to twenty two days after ovulation: observations on fresh specimens. *Journal of Anatomy*. 135: 191-209.

5 **Bollwein H., Mayer R. & Stolla R. 2003**. Transrectal Doppler sonography of uterine blood flow during early pregnancy in mares. *Theriogenology*. 60(4): 597-605.

6 **Bollwein H., Weber F., Woschée I. & Stolla R. 2004.** Transrectal Doppler sonography of uterine and umbilical blood flow during pregnancy in mares. *Theriogenology*. 61(2-3): 499-509.

7 **Carnevale E.M. & Ginther O.J. 1992.** Relationships of age to uterine function and reproductive efficiency in mares. *Theriogenology*. 37(5): 1101-1115.

8 **Denker H.W. 2000.** Structural dynamics and function of early embryonic coats. *Cells Tissues Organs*. 166(2): 180-207.

9 **Diskin M.G. & Morris D.G. 2008.** Embryonic and early foetal losses in cattle and other ruminants. *Reproduction of Domestic Animals*. 43(2): 260-267.

10 **Ford S.P. 1985.** Maternal recognition of pregnancy in the ewe, cow and sow: Vascular and immunological aspects. *Theriogenology*. 23: 145-159.

11 **Ford S.P., Chenault J.R. & Echternkamp S.E. 1979.** Uterine blood flow of cows during the oestrous cycle and early pregnancy: effect of the conceptus on the uterine blood supply. *Journal of Reproduction and Fertility*. 56(1): 53-62.

12 **Ford S.P. & Christenson R.K. 1979.** Blood flow to uteri of sows during the estrous cycle and early pregnancy: local effect of the conceptus on the uterine blood supply. *Biology of Reproduction*. 21(3): 617-624.

13 **Ford S.P., Christenson R.K. & Ford J.J. 1982.** Uterine blood flow and uterine arterial venous and luminal concentrations of estrogens on days 11, 13, and 15 after estrus in pregnant and nonpregnant sows. *Journal of Reproduction and Fertility*. 64(1): 185-190.

14 Gastal M.O., Gastal E.L., Kot,K. & Ginther O.J. 1996. Factors related to the time of fixation of the conceptus in mares. *Theriogenology*. 46(7): 1171-1180.

15 Ginther O.J. 1983. Mobility of the early equine conceptus. *Theriogenology.* 19(4): 603-611.

16 Ginther O.J. 1983. Fixation and orientation of the early equine conceptus. *Theriogenology*. 19(4): 613-623.

17 Ginther O.J. 1985. Dynamic physical interactions between the equine embryo and uterus. *Equine Veterinary Journal.* 3(Suppl 1): 41-47.

18 Ginther O.J. 1992. *Reproductive biology of the mare, basic and applied aspects.* 2nd edn. Cross Plains: Equiservices Publishing, pp.1-40 and 173-290.

19 Ginther O.J. 2007. *Ultrasonic Imaging and Animal Reproduction: Color-Doppler Ultrasonography* - Book 4. Cross Plains: Equiservices Publishing, 752p.

20 Ginther O.J., Gastal E.L., Gastal M.O. & Beg M.A. 2005. Regulation of circulating gonadotropins by the negative effects of ovarian hormones in mares. *Biology of Reproduction*. 73(2): 315-323.

21 Ginther O.J. & Silva L.A. 2006. Incidence and nature of disorientation of the embryo proper and spontaneous correction in mares. *Journal of Equine Veterinary Science*. 26: 249-256.

22 Ginther O.J. & Utt M.D. 2004. Doppler ultrasound in equine reproduction: principles, techniques, and potential. *Journal of Equine Veterinary Science*. 24: 516-526.

23 Girling J.E. & Rogers P.A.W. 2005. Recent advances in endometrial angiogenesis research. *Angiogenesis*. 8(2)**:** 89-99.

24 Griffin P.G. & Ginther O.J. 1993. Effects of the embryo on uterine morphology and function in mares. *Animal Reproduction Science*. 31: 311-329.

25 Leith G.S. & Ginther O.J. 1984. Characterization of intrauterine mobility of the early conceptus. *Theriogenology*. 22(4): 401-408.

26 McDowell K.J., Sharp D.C., Grubaugh W., Thatcher W.W. & Wilcox C.J. 1988. Restricted conceptus mobility results in failure of pregnancy maintenance in mares. *Biology of Reproduction*. 39(2): 340-348.

27 Mclean J.M. 1987. Early embryo loss. *The Lancet*. 329: 1033-1034.

28 Mossman H.W. 1971. Orientation and site of attachment of the blastocyst: A comparative study. In: *The Biology of the Blastocyst.* Blandau R.J. (Ed). Chicago: Chicago Press, pp.27-48.

29 Mossman H.W. 1987. In: *Vertebrate Fetal Membranes.* New Brunswick: Rutgers University Press, pp.79-83.

30 Reynolds L.P., Magness R.R. & Ford S.P. 1984. Uterine blood flow during early pregnancy in ewes: interaction between the conceptus and the ovary bearing the corpus luteum. *Journal of Animal Science*. 58(2): 423-429.

31 Roberts R.M., Xie S. & Mathialagan N. 1996. Maternal recognition of pregnancy. *Biology of Reproduction*. 54: 294-302.

32 Sharp D.C. 2000. The early fetal life of the equine conceptus. *Animal Reproduction Science*. 60–61: 679-689.

33 Silva L.A., Gastal E.L., Beg M.A. & Ginther O.J. 2005. Changes in vascular perfusion of the endometrium in association with changes in location of the embryonic vesicle in mares. *Biology of Reproduction*. 72(3): 755-761.

34 Silva L.A. & Ginther O.J. 2006. An early endometrial vascular indicator of completed orientation of the embryo and the role of dorsal endometrial encroachment in mares. *Biology of Reproduction*. 74(2): 337-343.

35 Silva L.A. & Ginther O.J. 2010. Local effect of the conceptus on uterine vascular perfusion during early pregnancy in heifers. *Reproduction.* 139(2): 453-463.

36 Silva L.A., Klein C., Ealy A.D. & Sharp D.C. 2011. Conceptus-mediated endometrial vascular changes during early pregnancy in mares - An anatomic, histomorphometric and vascular endothelial growth factor-receptor system immunolocalization and gene expression study. *Reproduction*. [in press].

37 Vanderwall D.K. 2008. Early Embryonic Loss in the Mare. *Biology of Reproduction*. 28: 691-702.

38 Weber J.A., Freeman D.A., Vanderwall D.K. & Woods G.L. 1991. Prostraglandin E_2 secretion by oviductal transport-stage equine embryos. *Biology of Reproduction*. 45(4): 540-543.

39 Woods G.L., Baker C.B., Baldwin J.L., Ball B.A., Bilinski J., Cooper W.L., Ley W.B., Mank E.C. & Erb H.N. 1987. Early pregnancy loss in brood mares. *Journal of Reproduction and Fertility*. 35(Suppl 1): 455-459.