Review Article Compte rendu

Fetal well-being assessment in bovine near-term gestations: Current knowledge and future perspectives arising from comparative medicine

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Abstract — Cloning technology is associated with multiple losses throughout pregnancy and in the neonatal period. Any maternal or fetal disease can compromise pregnancy. A paucity of data are available on bovine fetal well-being in late pregnancy; development of well-being assessment methods might augment early diagnosis of abnormal pregnancy or fetal distress, allowing early intervention. This review presents the current knowledge on fetal well-being based on bovine, ovine, equine, and human studies, as well as interesting research parameters that have been studied in other species and not yet investigated in cattle. Transabdominal ultrasonography allows for diagnosis of large placentomes and hydrallantois that frequently accompany clone pregnancies. Fetal inactivity or large hyperechoic particles imaged within the fetal annexes are associated with fetal distress or death, and should be reassessed to confirm compromised pregnancy. Measurements of different fetal parameters (thoracic aorta, metacarpal or metatarsal thickness) could be reliable tools for early detection of the large offspring syndrome commonly found in cloned calves.

Résumé — Le clonage bovin s'accompagne de pertes périnatales et en fin de gestation. Toute affection materno-fœtale peut compromettre la gestation. Les données disponibles sur l'évaluation du bien-être fœtal en fin de gestation sont parcellaires mais permettraient d'anticiper les gestations compromises afin de prendre les décisions appropriées. Cette revue de littérature se base sur les connaissances actuelles du bien-être fœtal bovin, ovin, équin et humain, ainsi que sur les paramètres prouvés intéressants chez les différentes espèces mais n'ayant pas encore été validés chez les bovins. L'évaluation échographique des annexes fœtales permet d'anticiper une augmentation de taille des placentomes qui accompagne certaines gestations clonées. L'hydroallantoide, une complication des gestations de clones peut être dépisté par une augmentation de la quantité de liquide allantoïde et la difficulté de visualiser le fœtus. Des débris en grande quantité dans les liquides fœtaux, une inactivité fœtale sont compatibles avec une gestation compromise et nécessitent de répéter l'évaluation de la gestation. La mesure échographique de l'aorte thoracique fœtale, de la largeur du métacarpe ou métatarse sont des indices potentiels de suivi de la croissance en fin de gestation qui permettraient d'anticiper le syndrome du gros veau.

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Introduction

Delivery of healthy offspring is the ultimate goal of a good breeding program, but in order to maximize reproductive efficiency, a number of assisted reproductive technologies (ART), such as multiple ovulation, embryo transfer, and, more recently, *in-vitro* production (IVP) embryo technologies have been used. While expensive and time consuming, ART enable the production of valuable offspring from genetically superior animals.

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Animal cloning, the latest ART, is used for genetic improvement (1), species conservation (2,3), and transgenic animal production for biomedical purposes (4). Notwithstanding the potential advantages of ART to the bovine industry, pregnancies derived from IVP and cloning technologies have been associated with health problems in newborns and surrogate dams (5–11). The large offspring syndrome (LOS), which is characterized by an increased length of pregnancy, fetal oversize, and increased placentome size, has been reported in IVP calves (5–7,9,10). In addition, hydrops of the fetal fluids (hydrallantois, hydramnios) has commonly been reported in cloned pregnancies (5,9).

Disease of the dam also may have an impact on the fetus (12–17). In contrast with LOS, placental insufficiency may result in intrauterine growth retardation and premature calving (17). Maternal stress may also initiate parturition, even if the calf is not fully prepared for extrauterine life. In situations where the dam suffers from a disease with a poor prognosis, it would be helpful to establish if the fetus is fully prepared for birth. Management of these near term high-risk pregnancies is a challenging task for the veterinarian.

The early stages of pregnancy are monitored to some degree during preventive medicine visits (18,19); however, little is known of the assessment of the bovine fetus and uterus during the final stages of pregnancy. In humans (20) and horses (21,22), ultrasonographic biophysical profiles have been described and are currently used in practice. This noninvasive technique detects abnormalities in the placental annexes and in the fetus before birth. High-risk pregnancies can be managed by referring the dam to a neonatal intensive care unit to manage the compromised neonate.

This article reviews techniques to assess fetal well-being and emphasizes the different elements that could be included in a biophysical profile to assess fetal viability and health status in the last trimester of pregnancy in cattle.

Review of the literature

The review was based on the following keywords: fetal well-being, fetal well-being and veterinary, fetal movements, fetal movements and veterinary, fetal distress, fetal distress and ultrasonography, fetal growth and ultrasonography, ultrasonography and late pregnancy, biophysical profile, and lamellar body count. The databases used were Medline and CAB Abstract. Relevant articles were included for review if they were applicable to bovine late pregnancy, done in other large animals (sheep or horses), or used by gynecologists or obstetricians. All studies of fetal well-being assessment in cattle and published in peer-reviewed journals were included, with the exception of endocrine profile studies in the dam or the fetus. The emphasis was on noninvasive methods to assess fetal well-being.

Assessment of the fetal annexes

During the last trimester of pregnancy, the bovine conceptus can be evaluated indirectly through its annexes (amniotic and allantoid fluid and placental membranes). In contrast to mares (22) and ewes (16), which have been used as animal models for human research, only 1 report of ultrasonographic studies of placental abnormalities during late pregnancy in cattle has been published (23). Experimental models of intrauterine growth retardation (IUGR) in ewes resulted in a decreased placental mass associated with growth-retarded lambs (24). Some of the experimental conditions that caused IUGR in ewes may be found in cattle, such as overfeeding during the growing period (25) and severe malnourishment during the 2nd half of pregnancy (26). The findings in IUGR in sheep suggest that placental ultrasonography and the size of the placentomes may be of use in assessing abnormalities during late pregnancy in sick cows. The growth retardation observed in fetuses of overfed primiparous ewes has been attributed to a primary reduction of placental growth (25). Anomaly of the uteroplacental unit has also been shown to be an important factor in the assessment of fetal well-being in diseased mares (13,22); however, the diffuse placentation of the equine species is different from the cotyledonary placentation of ruminants. Although information about ruminant placental characteristics is limited, visual comparison of expelled placentas from *in vitro*- and *in vivo*-produced calves with those from calves born from natural conceptions revealed larger placentomes in the *in vitro*-produced calves (9,27,28). Large placentomes may be the result of compensatory placental growth following placental insufficiency in the 1st trimester of pregnancy (27). Compensatory growth of remaining placentomes was experimentally induced in sheep after caruncles had been surgically removed during early pregnancy (29). Ultrasonography of the near term fetus and placenta in cows carrying normal and cloned fetuses showed an increase of the mean size of placentomes at 232 d of pregnancy in clone pregnancies (25.5 cm^2) compared with the control (17 cm^2) (23).

Hydropic conditions of the bovine uterus are common in clone pregnancies (5–7,9–11,23,27,30). Hydrallantois, the most common hydropic condition in cattle (31,32), is related to placental abnormality. In contrast, hydramnios is associated with congenital fetal abnormalities (31,32). Macroscopic changes include abdominal distension of the dam, increased fluid in the affected placental compartment, and thickening of the allantoic and amniotic membrane (32). With the exception of the description of the difficulties in observing the fetus in hydrallantois in ultrasonographs (23), no precise ultrasonographic descriptions of these conditions have been reported.

Decreased fetal fluid (oligohydramnios) is also considered to be an abnormal finding in humans (12,20,33) and horses (22,34). In humans, ultrasonographic measurement of the depth of amniotic fluid is used to detect oligohydramnios, which is frequently associated with IUGR, intrapartum asphyxia with increased frequency of deglutition of the fetus, and fetal demise (33). In the equine biophysical profile created by Reef et al (22), a decrease in fetal fluid depth was significantly associated with a poor outcome for the foal (22,34). Based on these findings, transabdominal ultrasonography might be an important tool with which to diagnose fetal fluid abnormalities.

Abnormal echogenicity of amniotic fluid has been described with various fetal diseases in foals, such as placentitis, septicemia, and the peripartum asphyxia syndrome; however, abnormal echogenicity also occurred in an apparently normal pregnancy, indicating that it may not be an important prognostic parameter (22). The amniotic and allantoid fluid in normal pregnancies in horses and humans may contain free-floating particles composed of desquamated cells or urinary crystals (35,36) and, in horses, may resemble hemorrhage or meconium. In ruminants, debris is not seen in normal pregnancies and, if observed repeatedly, may indicate a compromised or dead fetus (37).

Amniotic fluid can be sampled to predict fetal lung maturity. In cattle and humans, the lecithin to sphyngomyelin (L/S) ratio is correlated with the fetal production of pulmonary surfactant (33,38,39). Lecithin and sphyngomyelin are phospholipids that are transferred from the fetal lung to the amniotic cavity: the concentration of sphyngomyelin in amniotic fluid is stable, whereas that of lecithin increases during the final stages of pregnancy, causing an increase in the L/S ratio (38). Low amniotic L/S ratios at birth are associated with a poor outcome for bovine neonates. Bovine neonates in a high-risk pregnancy may develop respiratory problems very similar to those of respiratory distress syndrome in the human infant (38,39). Determination of the L/S ratio is time consuming and not routinely performed in most veterinary laboratories. Consequently, obstetricians have recently developed a rapid screening test to assess lung maturity based on the amniotic lamellar body count (LBC) (40,41). Lamellar bodies are produced by type II alveolar cells and are present in surfactant in increasing quantity as gestation advances (42). The concentration of lamellar bodies in amniotic fluid slowly increases as fetal breathing movements (FBM) increase (41–44). Due to the similarity in size between lamellar bodies (0.2–2 μm) and platelets (1–3 μm), quantification may be performed by standard electronic platelet cell counters. This screening test is highly valuable in human neonatology (43,45), but it has yet to be evaluated in other species. Although amniocentesis is considered to be safe in women, with complications in fewer than 1% of cases (33), it is a riskier procedure in late pregnancy in the bovine and equine species, with 8% and 25% abortion rates, respectively (46,47).

Assessment of the fetus

Assessment of the near-term fetus has the potential to be a reliable and consistant tool to detect fetal abnormalities; however, little information is available (13,20,22,23). In horses, the fetal biophysical profile includes fetal heart rate (FHR), fetal breathing movements (FBM), gross fetal body movements (FM), measurements of fetal aortic diameter, and fetal eye size (13,22).

Disease of the dam may cause a reduction in uterine blood flow and, subsequently, placental insufficiency (14,48). The hypoxemic fetus has bradycardia and decreased variability of heart rate, and decreased FM and FBM (49). In humans, the complexity of FM, fetal behavior, FBM, heart rate variability, and increased FHR following a period of activity are used as markers of fetal well-being and in the assessment of neurologic development (49–51). Fetal behavior defined on the basis of FHR patterns, fetal gross body movements, and fetal eye movements may be classified in 4 fetal states: quiet sleep, active sleep, quiet awake, and active awake (51). However, in a recent review, Danish obstetricians pointed out that there is no absolute definition of what is decreased fetal movement in humans (52). According to Olesen and Svare (52), scientific evidence that antepartum fetal surveillance improved perinatal outcome is inconsistent (52). In horses, lack of fetal movement during the examination of 2 mares was associated with a negative outcome (22). However, equine fetal sleeping periods, manifested by long periods of inactivity, have been reported without fetal demise (34,53). The duration of fetal inactivity was usually less than 30 min but infrequently more than 1 h (53). In humans, the fetus has frequent periods without gross movements. Activity occupies 9% to 18% of the time in late pregnancy and is observed more frequently in the afternoon than in the morning (53). Therefore, the equine fetus seems to be more active than the human fetus, in which periods of sleeping have been more frequently reported. This suggests that decreased fetal movements could be a reliable predictive factor of neonatal anomaly and should be reassessed for reliablility as a predictor of fetal health in the equine (22). In 25% of cases involving lack of FM, the foal was completely healthy; however, in 2 cases, sudden bouts of excessive activity were followed by abrupt cessation and death (34). In a more recent study performed over a 3-year period in 150 pregnancies examined monthly from day 150 to day 300, then weekly until birth, the investigators did not find a correlation between equine fetal movement and outcome of pregnancy (54). These findings show that, as in humans, decreased fetal movement should not be the only factor used to determine if the fetus is compromised. When fetal inactivity is observed, a reassessment of the pregnancy should be made.

In ewes, placental hypoxia created by decreasing uterine blood flow causes a rapid decrease in FM (55). Fetal breathing movements are seen as movements of the diaphragm, when fetal or maternal movements are absent (13,20–22), and they occur in 30% of sheep and human examinations during late gestation (36,49). In hypoxic fetal lambs, there is a transient decrease in the frequency of FBM, as is observed in humans (49,55). After a period of 12 to 16 h of hypoxic injury to the fetus, the frequency of FBM returns to normal, despite persistence of the hypoxic injury (56). By contrast, when fetal hypercapnia was combined with hypoxia, the frequency of FBM increased constantly (49). In 1 study, FBM were assessed in 150 equine pregnancies, but no association was made with fetal well-being or frequency of occurence (54).

There is limited information on FM and FBM in cattle (16,17,49). Fraser (57) used Doppler ultrasound and rectal palpation twice daily during the last week of pregnancy in heifers to evaluate fetal behavior. He observed that all calves showed decreased movements during the last 24 h before calving and that complex movements (more than 4 movement components at each isolated episode of activity) normally occur in late gestation, possibly resulting from a maturation of the nervous system. The depth of the uterus and the size of the calf may be limiting factors for fetal ultrasonography (37).

In horses (13,21,22,34,35), humans (12,20,36,49), sheep (48,58,59), and cattle (23,60–64) FHR is the most frequently reported parameter used to assess fetal well-being in the final stages of pregnancy. But interpretation of FHR is complicated (49) due to the multiple factors involved in its regulation: baroreceptor, chemoreceptor, sympathetic and parasympathetic nervous systems, and fetal activity (65). In sheep and humans, fetal bradycardia occurs in response to fetal hypoxia due to reflex distribution of cardiac output (49,66). However, in sheep, it is only transient and is followed by a period of tachycardia due to an increased level of catecholamines (49). Heart rate variability seems to be more reliable than FHR by itself in humans (20,49,66). Heart rate acceleration occurs in response to fetal movements, even if the fetus is hypoxemic (58). However, if hypoxic conditions are sustained, damage to the medulla oblongata and the midbrain is responsible for a loss of FHR variability, as demonstrated in sheep (58) and anencephalic human fetuses (67). Increased FHR can also be paroxysmal when hypoxic fetus shows epileptiform activity (58). In horses, the assessment of fetal well-being emphasizes the importance of HR rather than FHR variability (22,34). Decreased or increased FHR, compared with the FHR of normal pregnancy (68 to 82 beats/min) (21), is considered abnormal (22,34). Rantanen and Kincaid (68) consider bradycardia the most reliable indicator of impending equine fetal demise. A study involving 150 equine pregnancies monitored from day 150 to term, using ultrasonography, indicated that fetal cardiac rhythm was usually regular throughout pregnancy (54).

Various devices have been used to assess FHR in cattle: noninvasive techniques, such as fetal electrocardiography, transabdominal Doppler, and transabdominal ultrasonography (60–63), and invasive techniques, such as fetal aortic catheterization and uterine activity recording via myometrial electrodes (63). The conclusions were that the normal FHR during the last 2 wk of pregnancy is 105 beats/min (with a range of 90 to 125 beats/min) (61) and that FHR acceleration is associated with fetal movements (61); however, myometrial contractions did not significantly influence FHR (63). There is no easy technique to determine FHR variability and movement in cattle. Only periodic measurements of FHR can be performed with noninvasive procedures (ultrasonography or Doppler). Reef et al (22) proposed that 3 to 5 FHR measurements should be obtained during ultrasonographic recording sessions, preferably before and after fetal movements.

The assessment of fetal well-being is aimed at detecting any abnormality of the uterus, the fetal annexes, and the fetus. Any disturbance of one of the above may affect the others. A well recognized harmful effect of abnormal pregnancy in humans (12), foals (15,22), and lambs (16,25) is fetal growth retardation and low birth weight. Oversized newborns, a newer problem of fetal development associated with new reproductive technologies, such as cloning and *in-vitro* fertilization, has recently emerged (6,7,11,23,27,28). In humans and sheep, different measurements and their correlation with birth weight have been studied, including fetal head biparietal diameter and fetal abdominal circumference (12,69). Biparietal diameter and metacarpal bone length of lambs are suitable for estimating stage of gestation and birth weights (70). Growth curves have been obtained in humans and sheep by comparing these parameters to birth weight or fetal weight. Once retarded growth is anticipated, medical management can be adapted accordingly (69,70). The problem with large species is that it is not always possible to obtain these measurements due to abdominal depth, fetal position, or size of the bones. Reef et al (22) measured the systolic aortic diameter at the base of the heart and the thoracic width as possible indices of fetal size in the horse; aortic diameter was the only parameter that correlated to birth weight. This parameter was used in cattle by Chavatte-Palmer et al (23) to assess cloned and normal pregnancies; they found a significant correlation, but no significant difference, between gestational age and aortic diameter in clones and control calves, even if a statistical difference in birth weight was present (53 kg vs. 44 kg). Takahashi et al (71) showed a linear correlation between minimal metacarpal or metatarsal thickness and body weight measured in calves on the day of birth. The metacarpal and metatarsal dimensions were well correlated with the degree of dystocia in pregnancies that were obtained after artificial insemination. The same authors used metacarpal or metatarsal dimension, measured via transrectal ultrasonography, after determining the presentation of the fetus, to anticipate LOS in cloned pregnancy (72). These 2 studies indicated that these parameters could be included in a fetal biophysical profile in order to detect early LOS or dystocia.

Other parameters that have been suggested, but not fully validated, include fetal eye size, biparietal diameter, and length of the femoral bone (13). Bertolini et al (27) indicated that fetal eye size and area were predictive of bovine fetal growth. They also used foreleg length, crown-rump length, and humeral length as potential markers of bovine growth, but these could not be measured after 100 d of pregnancy. Moreover, the size of the fetal eye was of limited value after day 130 because of the reduction in eye growth in the last part of gestation (27).

Even if the above tools could determine whether a fetus has a normal growth rate, growth is different from maturity. Determining if the calf is fully adapted to extrauterine life is of critical importance to determine the outcome of the conceptus. Endocrinology is a promising tool for assessing fetal adaptation or well-being. Matsuzaki and Shiga (73) showed that cloned fetuses did not have a sufficient prepartum rise in plasma cortisol, leading to failure to initiate the switch to an adult mode in the insulin-like growth system and failure to initiate spontaneous parturition. An in-depth discussion about this topic is beyond the scope of this article.

Conclusion

There is a lack of well-defined studies to determine the healthy status of a bovine fetus during the last third of pregnancy. However, the widespread use of ART and the problems associated with *in vitro*-produced embryos and, in particular, cloned embryos (LOS, hydrallantois, respiratory distress, congenital anomalies) highlight the need for better procedures for the early detection of fetal distress or anomaly. Results obtained in other species (human, equine, and ovine) suggest that assessment of FHR, fetal activity, mean placentome size, and thickness of the allantoamniotic membrane should be included in the bovine fetal biophysical profile. However, more extensive studies are needed to increase the level of understanding of bovine late pregnancy. The ultimate goal of this knowledge, as in humans (74,75), is to decrease neonatal morbidity and mortality in low- and high-risk pregnancies. CVJ

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