Application of pregnancy associated glycoproteins (PAGs) to improve reproductive efficiency in cattle

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Key points

• Pregnancy diagnosis from a blood sample enables the detection of nonpregnant cows earlier than transrectal palpation after insemination
• Pregnancy diagnosis using PAGs is an efficient method that is based on detecting the presence of a pregnancy-specific protein
• PAG testing is commercially available in both blood and milk
• PAG testing may also provide a useful tool for detection of pregnancies that have a high probability of undergoing late embryonic mortality

Introduction

Increasing profitability of a beef or dairy herd is dependent upon increasing reproductive efficiency. In the U.S., the annual cost of reproductive failure to the beef and dairy industries is estimated to be $600 million and 1.4 billion, respectively, which is most likely a gross underestimate of the actual cost. The exact causes of reproductive failure include management issues, cow infertility, bull infertility, heat stress, and embryonic mortality. In order to increase reproductive efficiency there has been a rapid development and utilization of reproductive technologies (e.g. fixed–time artificial insemination [FTAI], estrus synchronization [ES], real-time ultrasonography, and chemical based pregnancy testing [PAGs]) that can improve both the reproductive management and genetic merit of a cattle herd. In fact, Seidel1 stated: “ES and AI are among the most powerful and applicable technologies for genetic improvement of a beef herd.” However, the primary challenge to adopting these technologies has been the commitment of time and labor associated with their implementation. Traditional methods of ES and AI require monitoring estrous behavior at least twice daily during the synchronization process. This traditional requirement of estrous detection is not only tedious and time consuming, but it also eliminates the insemination of animals that are either anestrus or not detected in estrus. Consequently, ES protocols that specifically synchronize the timing of ovulation in relation to the time of semen deposition have eliminated the need for estrus detection while achieving pregnancy rates similar to estrous detection-based ES protocols. Therefore, the adoption of FTAI by producers has increased. Based on the rate of FTAI adoption, it has been clear that these technologies are practically useful in increasing reproductive efficiency in cattle.2 The bottom line is that these protocols work effectively at synchronizing ovulation for appointment breeding with acceptable pregnancy rates. The question is: “How can we as producers further increase pregnancy rates to a single insemination or increase reproductive efficiency?” The answer to that question is not so easily resolved and it has prompted further investigations of how to increase dominant follicle maturity, increase oocyte competence, improve the uterine environment and promote placental health, to name a few. Our group has chosen to explore the use of PAGs (chemical based pregnancy testing) to increase reproductive efficiency in cattle.

Maternal circulating of PAGs as tools for reproductive management in cattle

PAGs and pregnancy establishment

Members of the modern PAG family are detectable in the maternal circulation by multiple tests (e.g. RIA and ELISA) starting soon after the time of binucleate cell formation (day 19-20 of gestation)3 until a few weeks after parturition.4,5 Circulating concentrations of bovine PAGs can be influenced by a number of factors including breed, weight, parity status of the dam, fetal sex, fetal number, and fetal birth
weight, along with pregnancy stage and status. However, the role that PAGs play during gestation remains undefined.

A majority of the work on PAGs has focused on the development of a reliable tool for diagnosing pregnancy in multiple ruminant species including cattle, sheep, goats, buffalo, bison, moose, and elk. Pregnancy associated glycoproteins are unique compared to other biochemical methods of pregnancy detection in cattle because these proteins are pregnancy specific. Pregnancy associated glycoprotein 1 (PAG1; also known as pregnancy specific protein B; PSPB) has been the primary PAG of most interest in relation to early pregnancy diagnosis because of the ability to detect PAG1 in the maternal circulation throughout gestation. However, Green highlighted two disadvantages in using PAG1 for pregnancy detection: 1) pregnancy diagnoses in the first month of pregnancy could be compromised due to the low and variable circulating concentrations of PAG1, and 2) the long half-life of these proteins (~8 days) in the maternal circulation after partition or fetal loss. Due to these concerns, there has been interest in detecting other PAGs for pregnancy detection. Green reported the establishment of an ELISA based test for early pregnancy PAGs with a relatively short half-life (4.3 day). It has also been shown that PAG concentrations first significantly increase in circulating around day 24 of gestation followed by a transient rise out to partition in cattle (Figure 1A) which is similar to that of other small ruminants (Figure 1B).

In the preceding study, PAGs were detected in all cattle by d 28 of gestation, PAG concentrations peaked around the time of parturition. After parturition PAGs were undetectable by eight weeks postpartum in 38 of the 40 cows, thus concluding that choosing different PAGs helps overcome the persistence of PAG immunoreactivity far into the postpartum period. In similar studies, after induced embryonic mortality, the half- life of circulating concentrations of PAGs was determined to be 35.8 ±21.9 h (mean±SD; Figure 2). These differences in PAGs half-life are presumably a result of distinct forms of the PAG family present earlier in gestation compared to term or a result of different clearance mechanisms between early and late pregnancy.

There are currently three commercial PAG testing platforms available for use, 1) BioPRYN (BioTracking, LLC, Moscow, ID), 2) DG29 (Conception Animal Reproduction Technologies, Beaumont, QC), and IDEXX Bovine Pregnancy Test (IDEXX Laboratories, Inc. Westbrook, ME). Current PAG assays have been documented to accurately diagnosis pregnancy in cattle with an average accuracy ranging from 93 to 96% in both blood and milk.

PAGs as a predictor of late embryonic mortality and as a biochemical marker for placental function

In cattle, the incidence of late embryonic/early fetal loss around the time of embryo uterine attachment is approximately 4 to 10%. The mechanisms associated with reproductive loss around the time of placentation are unknown, but may be associated with inadequate placental development or function. Along with the ability to use PAG assays as tools for pregnancy-detection, PAGs may also serve as a marker for monitoring embryonic/fetal viability along with placental function. For example, beef cows that successfully carried a pregnancy past day 72 of gestation had higher circulating concentrations of PAGs on day 28 compared to cows that exhibited late embryonic/fetal mortality between day 28 to 72 (using a sandwich ELISA). In the preceding studies, all cows had an embryo with a heartbeat on day 28 of gestation; however, cows that experienced late embryonic/fetal mortality after day 28 and before day 72 had decreased circulating concentrations of PAGs on day 28 (Figure 3). Similar data have been reported in dairy cows and sheep in which circulating concentrations of PAGs were higher or lower in animals that maintained or lost a pregnancy, respectively. However, Ricci reported that PAGs were not predictive of late embryonic mortality in dairy cattle. The preceding discrepancy in the efficacy of utilizing circulating concentration of PAGs on day 28 to 30 to predict late embryonic mortality in cattle may be explained by the specific PAG assay that was employed. The studies above that reported an association between circulating PAG concentrations and embryonic mortality utilized an antisera directed against placentomes collected during early gestation and developed by Jon Green that has been shown to accurately predict late embryonic mortality in beef and dairy cattle. Thompson also reported circulating concentrations of PAGs on day 30 were lower in dairy cows that experienced late embryonic mortality. In that study, circulating concentrations of PAGs were measured...
with a sandwich ELISA validated by Green\textsuperscript{5} and the antisera in that ELISA was also raised against PAGs secreted during early-mid gestation. In addition, there seems to be no correlation between embryonic size (crown rump length), embryonic width or embryonic volume at day 35 or 56 of gestation in beef cattle suggesting that these lower concentrations of PAGs in the maternal circulation are not purely reflective of a smaller embryo.

In cattle the use of somatic cell nuclear transfer (SCNT) usually results in high levels of fetal loss that occur throughout gestation. These losses are thought to be the result of abnormal placental development with lower placentome numbers.\textsuperscript{25,26} Hashizume\textsuperscript{27} reported that placentomes from SCNT pregnancies were decreased in number and that expression of PL and PAG genes was reduced compared to control pregnancies. There was a significant difference at d 35 in PAG secretion between control and SCNT pregnancies\textsuperscript{28} along with reports of significant increases in PAG concentrations in recipient SCNT cows at d 35\textsuperscript{25} and d 50\textsuperscript{29} of gestation that aborted during the first trimester. This increase in PAG concentrations was speculated to be from one of the following: 1) placental hypertrophy with a greater percentage of binucleate cells, 2) an increase in the synthetic activity of binucleate cells, or 3) a higher degree of PAG glycosylation which leads to an increase in their half-life in the maternal circulation.\textsuperscript{30} However, Constant\textsuperscript{30} reported that the increase in maternal concentrations of PAGs in abnormal SCNT pregnancies was not the result of any of the above and concluded that it was most likely due to an augmentation of PAG half-life, but speculated that it was not mediated through a higher degree of glycosylation.

Summary
Overall, using a biochemical marker such as PAG in ruminant ungulates may provide a powerful technique for a producer for identifying pregnant animals along with selecting cows that are most likely to experience embryonic/fetal loss thus increasing reproductive efficiency.
Figure 1. Circulating concentrations of PAGs during gestation in cattle (A) and sheep (B). Adopted and modified from Green/Wallace.5,31
Figure 2. Half-life of circulating concentrations of PAGs after induced embryonic mortality (0h). Modified from Pohler.11

Figure 3. Serum concentrations of PAGs in samples collected on day 28 of gestation from pregnancy cows with a viable embryo based on fetal heartbeat. Cows were then divided into whether they maintained pregnancy until day 72 of gestation (Embryonic survival) or embryonic mortality (between day 29-72). Modified from Pohler.11
References
