

Pregnancy-associated glycoproteins and pregnancy wastage in cattle

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Abstract

Accurate diagnosis of non-pregnancy and prompt re-enlistment of “non-pregnant” cattle into an appropriate breeding protocol are essential components of successful reproductive programs. Various methods aimed at improving detection of pregnancy and identification of non-pregnant cows earlier and more accurately are the focus of previous review articles and beyond the scope of this manuscript. Recently, the ability to measure pregnancy-associated glycoproteins (PAGs) in cattle has changed how pregnancy and, more importantly, non-pregnancy are detected. This presentation provides an overview of current research on the pregnancy-associated glycoprotein family, and how these glycoproteins might be utilized as indicators of pregnancy wastage in cattle.

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1. Introduction

Reproductive inefficiency in cattle can have devastating effects on economic success in dairy and beef units, where revenue is directly dependent upon reproduction or its associated effects on milk production. In dairy herds, the largest source of lost income as a result of reproductive waste or lost pregnancies is more days open, resulting in fewer days at peak milk production. It has been estimated that each pregnancy lost results in an average of \$US 640.00 lost income [1]. A Pennsylvania report utilizing an economic spreadsheet model to determine optimal breeding and replacement decisions for dairy cattle calculated the cost per day open beyond 60 days in milk to be as high as \$3 [2]. Pregnancy loss in beef herds negatively impacts economic returns, as herd income is heavily

dependent on the number of calves sold. Retention of non-pregnant beef cows, which continue to consume feedstuff, is fiscally nonsensical.

There is evidence suggesting a decline in bovine fertility over the last 50 years [3]. In dairy cattle, conception and pregnancy rates have decreased considerably as milk yield has increased [4,5]. Potential causes of bovine infertility include ovulation or fertilization failure, embryonic death, and fetal loss.

The etiologies of pregnancy loss in cattle are often divided into infectious and noninfectious categories. Bacteria, viruses, fungi and protozoa are potential infectious sources of conceptus loss, whereas toxins, genetic defects and stress (e.g. heat stress) can be noninfectious inducers of pregnancy loss. A review of infectious and noninfectious causes of pregnancy failure in cattle is beyond the scope of this paper and readers are directed to recent reviews [6,7] and another presentation at this meeting (Infectious Causes of Embryonic and Fetal Mortality), given by Dr. M. Daniel Givens.

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Effective strategies to improve bovine reproductive efficiency aim to reduce the number of days cows are not pregnant. A key component in reproductive management is accurate identification of open cows following insemination, thereby facilitating earlier enlistment into re-insemination programs. Veterinary practitioners utilize many methods to detect open cows, including transrectal palpation of the uterine contents, real-time ultrasonography, and detection of pregnancy-specific proteins in plasma. The objective of this review is to provide an overview of research to date on the pregnancy-associated glycoproteins (PAGs) and their potential role as indicators or monitors of pregnancy wastage in cattle.

2. Pregnancy wastage

The Committee on Bovine Reproductive Nomenclature [8] defined the embryonic period as the portion of gestation from conception to the end of the differentiation stage, approximately 42 days of gestation, and defined the fetal period as the portion of gestation from day 42 to the delivery of the calf. Pregnancy loss is more common during the embryonic period and becomes less frequent beyond day 50. Losses of pregnancy during the embryonic period may be divided into early embryonic death, which occurs prior to maternal recognition of pregnancy (MRP; in the cow, days 15–17 of the estrous cycle) and late embryonic death, which occurs between the time of MRP and the beginning of the fetal period (day 42).

Reproductive inefficiency due to either fertilization failure following insemination or pregnancy loss prior to maternal recognition of pregnancy and CL maintenance usually result in no alteration of the interestrus interval. Conversely, extension of the interestrus or interovulatory interval may indicate embryonic loss occurring around or after the period of CL maintenance [9,10]. Embryonic death at or after the time of CL maintenance can result in delayed luteal regression of at least 3 days after pregnancy loss [11], and extend the interestrus interval [10]. Humblot [10] suggested that luteolysis and return to estrus prior to day 24 might be linked with early embryonic loss, whereas an interestrus interval >24 days could indicate embryonic losses after day 16.

3. Pregnancy-associated glycoproteins

The pregnancy-associated glycoproteins, a multi-gene family belonging to the aspartic proteinase superfamily [12], are abundantly expressed in the

outer cell layer of the placenta of ruminants from the time the placenta attaches to parturition [13]. At least 21 bovine PAGs have been identified [14]. Pregnancy-associated glycoproteins are separated into two groups [13]. The group largely localized to the placental fetomaternal interface is considered of ancient origin and has been designated PAG-2. A separate subgroup expressed primarily in trophoblastic binucleate cells, considered to have resulted from a more recent series of gene duplications, has been designated PAG-1. In cattle, some PAGs from the PAG-1 subgroup become detectable in the maternal circulation beginning at approximately the time of implantation (~day 25). Concentrations of these glycoproteins steadily increased throughout pregnancy, peaking just before parturition. Pregnancy-associated glycoproteins are thought to function in immunomodulation, trophic support of the CL, and regulation of trophoblastic cell migration [13].

The most widely recognized work with PAGs has been the development and commercial application of pregnancy-specific protein B (PSP-B) as a biochemical marker of pregnancy [15]; BioPrynTM (BioTracking, LLC, Moscow, ID, USA) is a commercially available enzyme-linked immunosorbent assay (ELISA) designed to detect PSP-B [16,17]. Other groups have developed immunoassays for PAGs other than PSPB that are likely based on similar antigens [18–21], whereas in one report the antigen was called pregnancy-associated glycoprotein [19,21] and in the second it was named pregnancy serum protein-60 to denote its molecular weight [20], all are reported to have similar, if not identical, amino-terminal amino acid sequences [12,22] and all will be referred to as bovine PAG-1 (bPAG-1) for the remainder of this paper. Mean bPAG-1 concentrations in cattle begin to increase from days 15 to 35, but variation in serum bPAG-1 concentrations among cows precludes their use as a reliable indicator of pregnancy until days 26–30 [10,21].

Variations in inter-assay agreement of bPAG-1 occur and may be due to variations in the antiserum utilized. In two studies, one designed to determine factors affecting plasma bPAG-1 concentrations in pregnant high-producing dairy cows [23] and the other to assess the predictive importance of maternal bPAG-1 concentrations in recipients carrying somatic clones to pregnancy outcome [24], bPAG-1 concentration varied with the assay, and more specifically the primary antibody being used. It is likely that the different polyclonal antisera used in radioimmunoassay (RIA) systems to measure bPAG-1 recognize other PAG molecules, and because more than 100 genes encode

various PAG molecules in ruminant placenta [25], varying affinities would explain the inconsistent or varying inter-assay results. Assays utilizing heterologous bPAG-1 RIA systems may be more sensitive [26] and demonstrate higher bPAG-1 concentrations, improving ability to detect early pregnancy. Sensitivity and specificity were improved when a monoclonal antibody was used to detect bPAG-1 [18]. The monoclonal antibody detected only a few bPAG-1 family members belonging to the binucleate trophoblasts cell-specific group; use of the monoclonal antibody permitted pregnancy to be detected in all animals by 28 days after AI.

Persistence of bPAG-1 concentrations in the postpartum period or after pregnancy loss is one shortcoming of pregnancy determination based on bPAG-1. Managers and practitioners must be aware of this limitation of current testing technology. The proteins reached a maximum concentration in plasma at approximately the time of parturition [20,21,27]; with their long half-life, they remain in circulation for 2–3 months after calving [20,21,28]. When using plasma bPAG-1 for pregnancy diagnosis, the concentrations expected at various times following conception and previous parturition must be taken into account [21,29]. Cows <2 months postpartum may have bPAG-1 concentrations above the pregnancy/open cut-off value, even if they had not been inseminated. An important consideration is that bPAG-1 detected by monoclonal antibodies have a relatively shorter half-life, averaging only 4.3 days during the postcalving period, and bPAG-1 detected with monoclonal antibodies were below pregnancy threshold concentrations by 8-week postpartum [18] and 2.7–7.0 days after induction of embryonic mortality [30,31].

The profiles of bPAG-1 have been evaluated as a method to predict embryonic and fetal loss in ruminants [32–34] and specifically fetal viability in cattle [21,24,35–37]. Such studies require specific knowledge of the assay utilized, and the half-life of the measured PAG.

4. Fertilization and early embryonic losses

Early experiments [38,39] indicated that fertilization rates were usually high in cattle (85–95%), suggesting that embryonic death was responsible for much of the reproductive wastage. Based on more recent studies, fertilization rates may be somewhat lower. Fertilization rates averaged 75.0% in lactating [40] and 98.6% in nonlactating beef cows [41,42], whereas rates were similar between lactating and nonlactating dairy cows,

averaging 76.2 and 78.1%, respectively [43–45]. Therefore, although lactation may decrease fertilization in beef cattle, it is less important in dairy cows.

Embryonic death prior to maternal recognition of pregnancy (14–19 days after fertilization) may be 35–40% in the bovine [46,47]. In dairy cattle, within 5–6 days after insemination, only 65% of fertilized oocytes resulted in viable embryos [48]. Early embryonic survival varied considerably with parity and lactation status. Embryo viability 5–6 days post-insemination as a percent of fertilization for parous lactating, parous nonlactating, and nulliparous cattle was 50.0, 57.9, and 71.9%, respectively [48]. Early embryo viability in beef cows followed a similar pattern as in dairy cattle, except that rates were generally higher and comparable between parous nonlactating cows and nulliparous heifers [48].

As discussed previously, mean bPAG-1 concentrations in cattle began to increase from days 15 to 35. However, variation in serum bPAG-1 concentrations among cows precluded reliable use as an indicator of pregnancy until approximately days 26–30 [10,21]. Recommendations to delay diagnosis of pregnancy utilizing bPAG-1 until ≥ 30 days after insemination precluded their use in detecting early embryonic death.

5. Late embryonic death

Ultrasonography and other methods of early pregnancy diagnosis have enabled researchers to characterize the timing and extent of late embryonic losses in cattle. Between days 18 and 28, i.e. after maternal recognition of pregnancy but before traditional methods of pregnancy diagnosis of pregnancy are widely used, embryonic mortality may be 5–10% [49–52]. An additional 5–10% embryonic mortality may occur in lactating dairy cows from days 28 to 42 (late embryonic death) [48,53]. Humblot [10] evaluating embryonic losses in dairy herds in France, reported a late embryonic death rate of 14.7%. The incidence of late embryonic loss in dairy cows may vary widely and depend on milk production and heat stress. Late embryonic death after day 27 was 3.2% in dairy cows producing 6000 to 8000 kg of milk per year in Ireland [54]. However, when high-producing dairy cows were subjected to heat stress, late embryonic loss occurred in 42.7% of cows [55].

6. Fetal death

The incidence of fetal attrition can vary widely across geographical location, animal type, animal use,

management, and age. Fetal death is typically less prevalent than early and late embryonic losses. Beef and dairy cows experienced fetal losses of up to 11% [48], whereas fetal losses in primigravid beef and dairy cattle were relatively low (2.5–4.2%) [48]. Accurate determination and prediction of fetal loss could allow more rapid re-enlistment into a breeding protocol or culling, either of which might reduce losses due to abortion.

7. Research review

7.1. Spontaneous loss

Pregnancy-associated glycoprotein profiles in cattle with spontaneous or inadvertent embryonic and fetal loss have been the focus of several studies. Many of the experiments reported here were designed to assess the accuracy of pregnancy diagnosis by transrectal ultrasonography with the measurement of circulating bPAG-1 as a confirmation of pregnancy. One study [56] examined lactating dairy cows repeatedly between 27 and 59 days after insemination, utilizing transrectal ultrasonography for diagnosis of pregnancy and non-pregnancy, while concurrently measuring plasma bPAG-1 concentrations. In four cases of embryonic/fetal mortality, high concentrations of both bPAG-1 and progesterone confirmed the presence of a live conceptus and a functional CL at the time of the initial examination. Fetal death, determined by ultrasonography, was preceded by a decrease in plasma concentrations of bPAG-1 in all cases, and by a decrease in plasma progesterone concentrations in 3 of 4 cases.

Szenci et al. [29] evaluated three different methods of early pregnancy detection. Cows were successively examined for pregnancy by transrectal ultrasonography, PSPB and bPAG-1. Twelve of 138 cows (8.6%) experienced embryonic mortality, defined as the absence of an embryo or fetus previously detected by ultrasonography, or the absence a heartbeat in an ultrasonographically detectable embryo. Most embryonic loss occurred between 29 and 38 days after AI. Following embryonic mortality, plasma concentrations of PSPB and bPAG-1 decreased steadily. Due to the relatively long half-life (~7 days) in maternal circulation, both PSPB and bPAG-1 remained above the threshold for pregnancy several days following fetal death. Concentrations consistent with those in non-pregnant cattle were reached in eight and two cows, respectively, by 53–58 days after AI. The PSPB concentration was below the threshold for pregnancy diagnosis in all but one cow, 8–24 days after embryonic mortality. However, in the same time frame, bPAG-1

concentration was below the threshold for pregnancy diagnosis in only two of 12 cows experiencing embryonic loss.

The duration of the interval that progesterone, PSPB and bPAG-1 remained above the diagnostic value for luteal function or pregnancy following detected death of the conceptus was evaluated in 11 dairy cows between 26 and 58 days after AI [57]. In six cows, progesterone concentrations declined to <0.5 ng/mL within 0–9 days after diagnosis of death of the conceptus. In three cows, progesterone concentrations dropped steeply (but never below 0.5 ng/mL) between 12 and 23 days after late embryonic mortality (LEM). The PSPB concentration was at or below the cut-off value in 8 of 11 aborting cows within 0–20 days after LEM. Around the time of LEM or fetal mortality (FM), bPAG-1 concentrations began to decrease steadily and reached the cut-off value for pregnancy diagnosis in 4 of 11 cows within 58 days following AI.

Both PSPB and bPAG-1 concentrations declined in most LEM cases, whereas the CL still produced progesterone, consistent with a previous report [30]. Occurrence of LEM/FM was diagnosed on the basis of PSPB retrospectively in seven cows, as concentrations were less than the cut-off values during the examination period. In the case of bPAG-1, only four cows experiencing LEM/FM reached the cut-off value after conceptus death, suggesting a longer half-life for bPAG-1 than for PSPB.

In a study designed to establish whether bPAG-1 measurements during the early fetal period could be associated with early fetal loss, the pregnancies of 98 high-producing (11,450 kg per cow) dairy cows were monitored. Collection of blood samples (for bPAG-1 and progesterone concentrations) and transrectal ultrasonography were done on days 35, 42, 49, 56, and 63, or until pregnancy loss. Of the 98 pregnancies monitored, 18.4% suffered fetal loss. In this study, bPAG-1 and progesterone concentrations did not decline before fetus expulsion for any cow with pregnancy loss, and the concentrations were not significantly different between the no loss and the fetal loss groups. However, based on the odds ratio, the risk of fetal loss was 10 and 6.8 times more likely in cows with low (<2.5 ng/mL) and high (>4.0 ng/mL) bPAG-1 concentrations, respectively (on day 35), than in cows with medium bPAG-1 concentrations (2.5–4.0 ng/mL) used as a reference. Therefore, bPAG-1 concentration on day 35 might be used as a predictive tool for fetal loss. According to the authors this may have clinical application; a single sample on day 35 could provide more useful information than a series of samples from days 35 to 63 [58].

Profiles of circulating PAG concentrations in small ruminants experiencing pregnancy wastage have also been assessed. From 30 days after mating to parturition or abortion, weekly PAG concentrations were determined in 29 goats with normal pregnancies and 20 goats with various forms of pregnancy failure [59]. Two goats experienced pseudopregnancy, eight aborted a single fetus well before term, four had one live and one dead fetus near term, and six aborted after 154 days of pregnancy. The two pseudopregnant goats were diagnosed with hydrometra and PAG concentrations never rose above the threshold for pregnant animals. In all of the goats that experienced pregnancy wastage, pregnancy was initially confirmed by a rise in PAG concentration, followed by a substantial drop secondary to the death of a large portion of the placenta and binucleate cells. In all cases (pre-term abortions, abortion of a single fetus in a multi-fetus pregnancy, or abortion after a prolonged gestation) PAG concentrations decreased days to weeks before progesterone declined, which was followed by fetal expulsion 2–3 days afterward. The authors concluded that serial PAG analysis in pregnant goats was a better indicator of conceptus viability than progesterone concentrations, and that measuring PAG in circulation of pregnant goats may become a diagnostic tool to determine cause or timing of pregnancy wastage, or identify high-risk pregnancies in farm animals.

7.2. Induced pregnancy loss

7.2.1. Chemically induced abortion

Unlike spontaneous or inadvertent abortions in cattle, chemically induced abortions may be a predictable and repeatable model to assess circulating bPAG-1 profiles following pregnancy loss. A study using this model with pregnant heifers described the clinical features and plasma profiles of bPAG-1, prostaglandin metabolites and progesterone following chemically induced abortions with the prostaglandin analog, cloprostenol. Luteolysis, subsequent fetal death and abortion was induced from 63 to 116 days of pregnancy. Fetal death and then expulsion occurred from 2 to 4 days after treatment. Plasma bPAG-1 concentrations began to decrease after fetal expulsion at a rate dependent on the stage of pregnancy stage at which cloprostenol was administered. The rate of decrease seemed relatively slower as the stage of pregnancy increased. A half-life ranging from 1.8 to 6.6 days was estimated. In this model, the plasma concentration of bPAG-1 following fetal death decreased gradually; the rate of decrease varied with

the stage of pregnancy [60]. One shortcoming of this model was that most abortifacient agents affect the conceptus (placenta, embryo/fetus or both) and therefore may affect the profile of bPAG-1 prior to fetal death.

7.2.2. Infectious abortion

In cases of bovine abortion submitted to veterinary diagnostic laboratories, an etiologic diagnosis is determined only 23–45% of the time, with most attributed to infectious causes [6]. Other surveys [61,62] suggest an additional 17–25% of bovine abortions have lesions suggestive of an infectious cause, suggesting that >50% of bovine abortions may be attributed to infectious causes. Studies have been conducted to determine the effect of known infectious abortifacient agents on bPAG-1 profiles in pregnant cattle. Of the known infectious causes of bovine abortion, most infect the placenta, embryo/fetus or both. Because PAGs are synthesized and secreted by cells of the placenta and are indicators of placental health, examining the effects of infectious abortifacients on bPAG-1 profiles may help determine the site of disease/pathology and increase understanding of the mechanisms causing abortions.

In a study designed to investigate the profiles of circulating concentrations of PSPB and progesterone following late embryonic or early fetal mortality due to bacterial infection, pregnant (confirmed with PSPB and transrectal ultrasonography) lactating dairy cows were given either intrauterine inoculation of *Arcanobacter pyogenes* (treatment group; $n = 4$; 30–41 days after AI) or an IM injection of PGF_{2 α} (control group; $n = 4$; 50 days after AI). In both groups, plasma PSPB concentrations declined from the day of treatment, with the same half-life detected in postpartum cows (7 days) [16]. In the prostaglandin-treated group, progesterone concentrations declined rapidly, whereas in the *A. pyogenes* group, progesterone concentrations were maintained at concentrations typical of pregnancy for at least 20 days following infection [30].

LEM was induced by either trans-cervical inoculation with *A. pyogenes* ($n = 5$) or prostaglandin treatment ($n = 4$) in heifers on days 30–38 [31]. Inoculation with *A. pyogenes* caused LEM in 4 of 5 heifers. In heifers that aborted following inoculation with *A. pyogenes*, bPAG-1 half-life was 2.7–3.5 days, and concentrations declined to the pregnancy cut-off value (0.8 ng/mL) within 4–8 days post-inoculation. Three of four heifers treated with PGF_{2 α} experienced LEM 2–3 days after treatment. Beginning on the day of LEM, plasma bPAG-1 declined with a half-life of 3.2–3.9 days, and reached

the pregnancy/open cut-off value 5–8 days after treatment. The short half-life reported in this experiment was in contrast to previous reports [30] that PSPB had a half-life of 7 days. One possible explanation for the difference in half-lives is that different PAGs in circulation may have different rates of metabolism and excretion. Unlike bPAG-1 concentrations, progesterone declined erratically (if at all) in heifers inoculated with *A. pyogenes* (progesterone concentrations remained elevated for the entire experiment in one heifer that developed pyometra). Similarly, when embryonic mortality was induced by inoculation of *A. pyogenes* between days 35 and 41, progesterone concentration remained elevated for 20–40 days [63].

Neospora caninum has a high rate (up to 95%) of transplacental transmission in infected cows and is an important cause of bovine abortion and stillbirth worldwide. When pregnant cows not infected with *N. caninum* ($n = 6$) and non-aborting pregnant cows chronically infected with *N. caninum* ($n = 13$) were compared, there was no difference in plasma bPAG-1 concentrations [64]. Three chronically infected cows aborted, with abortion presumed to be the result of *N. caninum*. Two of the three failed pregnancies resulted in fetal mummification; bPAG-1 concentrations were low to undetectable when mummification was detected. The authors concluded that *N. caninum* infection failed to affect placental function in chronically infected cows not suffering from abortions, whereas in aborting cattle, bPAG-1 concentrations were useful as indicators of fetal-placental status [64].

Zarrouk et al. [65] conducted a retrospective study to relate PAG profiles of goats experimentally infected with microbes known to cause abortion. Circulating PAG concentrations were measured twice weekly before and after inoculation of pregnant goats with either *Toxoplasma gondii* ($n = 5$) or *Listeria monocytogenes* ($n = 8$). The *T. gondii*-infected group was inoculated subcutaneously with bradyzoites at 71 days after mating, whereas the *L. monocytogene*-infected group was inoculated IV on days 69–77 ($n = 4$) or days 105–106 ($n = 4$). A decline in circulating PAG concentrations was detected in all cases following inoculation with *T. gondii* and preceded fetal death. There was large variation in the interval from inoculation to decline in PAG concentration (2–24 days) and to abortion (55–74 days). In most goats infected with *T. gondii*, PAG concentrations decreased gradually until the day of fetal expulsion. By comparing the PAG profiles with ultrasonography data, it was determined that death of the fetus occurred weeks after the inoculation.

Seven of the eight goats inoculated with *L. monocytogenes* aborted and *L. monocytogenes* was isolated from the organs of each of the eight fetuses. Of the four goats inoculated at approximately day 70, three aborted 9 days later and one subsequently kidded. In goats that aborted, PAG concentrations decreased dramatically from the day of inoculation to abortion. All goats infected at approximately day 105 experienced a decline in circulating PAG concentrations from 1 to 8 days after inoculation, and aborted by 10 days after inoculation.

Major differences in PAG profiles were observed after infection with the two pathogens. The authors speculated that the change of PAG profiles reflected placental distress following the invasion by the microbes; furthermore, it was likely that destruction of the binucleate cells after infection caused the decline in PAGs. When goats were infected with *T. gondii*, abortion occurred 55–74 days after inoculation, with PAG concentrations declining gradually until the day of fetal expulsion. However, in goats inoculated with *L. monocytogenes*, PAG decreased dramatically from the day after inoculation to the occurrence of abortion, 9 days later. The different pattern of PAG might have been due to differing effects of the slow multiplication of tachyzoites of *T. gondii* in the placenta, compared to the more acute placentitis induced by *L. monocytogenes* infection, and the resultant rates of compromise of the binucleate cells. By comparing ultrasound data with PAG profiles, the authors concluded that the death of the fetus occurred days (*L. monocytogenes* group) to weeks (*T. gondii* group) after the change in placental function, as indicated by a reduction in PAG concentrations.

7.3. Abnormal offspring syndrome

Somatic cloning in cattle is associated with increased risk of fetal loss due to defects in formation or function of extra-embryonic membranes and developmental anomalies which may be manifested throughout pregnancy. This collection of fetal and placental abnormalities has recently been termed “Abnormal Offspring Syndrome” (AOS) [66]. There are no objective means to identify recipients carrying clones that will develop the syndrome. Chavatte-Palmer et al. [24] utilized repeated ultrasonographic observations of the fetus and placenta, as well as repeated measurement of maternal concentrations of bPAG-1 during the first 2 months of gestation in groups of pregnancies resulting from somatic cell nuclear transfer (SCNT) and pregnancies not the result of SNCT. These measurements were then related to pregnancy outcome and

occurrence of AOS. As measured by two different radioimmunoassays, concentrations of PAG were significantly higher in cows carrying clone (SCNT) pregnancies which went to term than *in vivo* derived pregnancies that went to term. Using the same two assays, bPAG-1 concentrations from days 34 to 50 were significantly lower in clone recipient cows suffering an early pregnancy loss than in those which maintained pregnancy to a later date. Therefore, measurement of bPAG-1 may be a practical non-invasive tool to follow pregnancy in clone recipients. Furthermore, primary growth retardation and abnormal placental function may precede abnormal fetal and placental growth at later stages of pregnancy.

8. Clinical relevance

Reproductive inefficiency is an important source of economic loss in dairy and beef production, and improved accuracy and sensitivity of pregnancy and non-pregnancy diagnosis can decrease financial losses by reducing days open. The measurement of circulating concentrations of bPAG-1 in cattle as a biochemical marker of pregnancy is well established. There is considerable evidence that changes in concentration of PAGs in pregnant ruminants may be both diagnostic and predictive of pregnancy loss. Practical application or translation of this technology to determine or predict abortion in cattle on farms is not yet realized. Currently pregnancy wastage is most commonly detected by repeated/serial transrectal examination of the uterus and its contents by transrectal palpation or ultrasonography. For measurement of bPAG-1 concentration in cattle to become more widely used for this purpose, it must have some advantage over current methodologies. To address the efficacy of evaluation of circulating bPAG-1 values as a method to predict or accurately identify cattle experiencing pregnancy loss, some studies have directly and/or indirectly compared bPAG-1 concentrations to the more traditional techniques in cattle experiencing pregnancy wastage.

Pregnancy status of lactating dairy cows 27 days after timed AI, as determined by plasma bPAG-1 ELISA [18] and transrectal ultrasonography, had a high level of agreement ($\kappa = 0.87\text{--}0.90$) [67], suggesting that ultrasonography was no better than measurement of bPAG-1 at detecting pregnancy at day 27. When comparing the bPAG-1 ELISA to transrectal ultrasonography, the rate of false negative results for pregnancy was less than the rate of false positives. Because the half-life of bPAG-1 in maternal circulation can range from 4.3 to 7.0 days during the postcalving period and

2.7–7.0 days after induction of embryonic mortality [30,31], the authors speculated that pregnancy loss may have accounted for some of the false positive results of the bPAG-1 ELISA. From the period of decreasing bPAG-1 concentration in maternal circulation after embryonic death to values lower than the cut-off point for pregnancy, bPAG-1 might be detected by ELISA, and lead to a false-positive diagnosis. Conversely and advantageously, transrectal ultrasonography can be used to visualize embryonic death and reduce the frequency of false positive results. If bPAG-1 concentrations decreased rapidly after embryonic death, the bPAG-1 ELISA might be used to detect losses earlier than transrectal palpation, due to the lag between embryonic death and expulsion of the conceptus [11,68]. Because embryonic viability can be assessed readily by transrectal ultrasonographic observation of embryonic and fetal heartbeat, the availability of ultrasonography eliminated any advantage gained by the assessment of circulating concentrations of bPAG-1.

Some researchers proposed that bPAG-1 may be useful as predictors of embryonic loss. A study [33] designed to develop a means to predict embryonic loss at the time of early pregnancy detection measured serum PSPB and progesterone concentrations 30–36 days post-insemination in lactating dairy cows. Over a 25-month interval, more than 8000 blood samples were analyzed from three farms; 50.3% were classified as pregnant cows, according to the BioPrynTM assay. When cows were examined by transrectal palpation 60 days after AI, a total of 710 late embryonic losses (17.4%) were detected. The authors predicted late embryonic loss would occur when a sample's optical density (OD) reading in the BioPrynTM assay was between 0 and 30% above the cut-off OD for pregnancy. Using this criterion, 31.8% of late embryonic losses were correctly predicted. However, when samples had both an OD value of 0–30% above cut-off OD for pregnancy, and serum progesterone concentrations were <2 ng/mL ("low"), 92% of late embryonic loss was accurately predicted. Although PSPB concentrations alone did not accurately predict late embryonic loss, in combination with "low" progesterone, PSPB concentrations 0–30% above cut-off had high predictive value. In addition to accurately predicting early embryonic loss, only measuring progesterone in samples with an OD from 0 to 30% above pregnancy cut-off greatly reduced the number of progesterone determinations and thus assay cost [33].

Following LEM, persistence of the CL may prolong the interestrus interval and increase the number of days open, leading to the need for the early diagnosis of

embryonic losses in cows. Based on many of the experiments summarized in this paper, decreasing bPAG-1 concentrations may indicate embryonic or fetal loss before progesterone concentrations decrease. Embryo mortality is most conveniently detected with transrectal ultrasonography. However, since blood sampling does not require the special equipment or skilled operators, detection of bPAG-1 in sequential samples could be a good alternative to identify late embryonic loss. Serial or single determination of bPAG-1 alone, or in combination with progesterone, may be superior to progesterone only, and provide an alternative to ultrasonography for detecting embryonic mortality in cattle.

A shortcoming for the use of bPAG-1 to determine pregnancy loss is their varied and often protracted half-lives. Another objection to measurement of bPAG-1 to determine pregnancy status and/or pregnancy loss is that concentrations of these proteins can vary for reasons other than pregnancy. López-Gatius et al. [23] demonstrated a significant negative correlation between milk production and bPAG-1 concentration measured on day 63 in high-producing dairy cows (average milk production = 11,450 kg/(cow year)) carrying live fetuses. Depending on the RIA used, each 1 kg increase in milk yield was associated with decreases of 0.08–0.1 ng/mL bPAG-1 concentration. The authors speculated that bPAG-1 concentrations may be lower in high-producing dairy cows as a result of increased bPAG-1 metabolism and clearance. Higher producing dairy cows consume more feed, resulting in increased liver blood flow and metabolism of gonadal steroids [69] and possibly PAGs. That PAGs have also been detected in milk of pregnant dairy goats [70] and postpartum cows [23] suggested a substantial amount of PAGs are excreted through the mammary gland into the milk, further confounding use of PAGs for determining pregnancy and pregnancy wastage.

9. Conclusion

Rapid and accurate diagnosis of non-pregnancy can reduce the negative economic impacts of pregnancy wastage in cattle. Early identification of “open” cattle allows prompt re-enlistment into appropriate breeding protocols or more timely culling to effectively reduce days open and financial losses. Another potential benefit to more rapid diagnosis of pregnancy loss is earlier identification of the cause(s), possibly mitigating further losses. The measurement of bPAG-1 in circulation of cattle as a biochemical marker of pregnancy is well established and the quantification of bPAG-1 in the

circulation of pregnant cattle may be a valuable research tool to elucidate the effects/targets of different infectious abortifacients, by indicating the wellbeing of the extra-embryonic tissue/placenta. At this time, the practical or routine use of bPAG-1 measurements to predict or diagnose pregnancy loss is limited by assay variability (depending on antibody used and specific PAG(s) detected), protracted half-lives of bPAG-1 following pregnancy loss and parturition, and the effect of variables other than pregnancy status (e.g. milk yield) on circulating concentrations of bPAG-1 in cattle. As more specific assays designed to detect particular classes or individual bPAG-1 become available, pregnancy-associated glycoprotein may become more widely and routinely used for diagnosing pregnancy wastage in cattle.

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