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SIRE CONTRIBUTION TO PREGNANCY ESTABLISHMENT AND MAINTENANCE IN BEEF COWS

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To the Graduate Council:

I am submitting herewith a thesis written by Gessica Araujo Franco entitled "SIRE CONTRIBUTION TO PREGNANCY ESTABLISHMENT AND MAINTENANCE IN BEEF COWS." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Ky G. Pohler, Major Professor

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SIRE CONTRIBUTION TO PREGNANCY ESTABLISHMENT AND MAINTENANCE IN BEEF COWS

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DEDICATION

To my grandma. I will miss you until we meet again!

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ABSTRACT

Pregnancy loss is a major component of reproductive inefficiency and economic loss in both the beef and dairy industry. Research tends to focus on female contribution to pregnancy maintenance and overlook the role of the sire, especially beyond early embryonic development stages. The aim of this study was to identify sires associated with high or low pregnancy loss and to investigate their effect on placental function, using circulating concentrations of pregnancy-associated glycoprotein [PAGs]. For the first part of the study, multiparous beef cows were randomly timed artificially inseminated [FTAI] with semen from either of 6 Angus sires of proven fertility and later, sires were retrospectively classified according to amount of pregnancy loss between days 30 and 100 of gestation. Pregnancies sired by high pregnancy loss sires had lower (P=0.05) circulating PAG concentration compared with pregnancies sired by low embryonic loss. For the second part, cows were artificially inseminated with either Nelore or Angus sires. Cows receiving semen from Nelore sires had greater ($P < 0.001$) pregnancy rate, greater ($P = 0.014$) pregnancy loss and lesser ($P = 0.002$) PAG concentrations at day 30 of gestation compared with cows receiving Angus semen. Estrus expression were evaluated in all cows using detector patches. Cows that expressed estrus prior to FTAI had higher pregnancy rate at day 30 and higher odds of maintaining pregnancy up to day 100 of gestation. Moreover, the effect of estrus in pregnancy rates was highly variable between sires. In summary, PAG concentrations reflected probability of pregnancy maintenance and were

influenced by both sire and sire breed used at FTAI. A large variation in the incidence of pregnancy loss was detected among sires that could not be predicted with standard semen fertility evaluations. Exploring the relationship of sire and PAG production might be promising to improve sire selection with regard to pregnancy loss.

PREFACE

Ando devagar
Porque já tive pressa
E levo esse sorriso
Porque já chorei demais
Hoje me sinto mais forte
Mais feliz, quem sabe
Só levo a certeza
De que muito pouco sei
Ou nada sei

Conhecer as manhas
E as manhãs
O sabor das massas
E das maçãs
É preciso amor
Pra poder pulsar
É preciso paz pra poder sorrir
É preciso a chuva para florir

Penso que cumprir a vida
Seja simplesmente
Compreender a marcha
E ir tocando em frente
Como um velho boiadeiro
Levando a boiada
Eu vou tocando os dias
Pela longa estrada, eu vou
Estrada eu sou

Todo mundo ama um dia
Todo mundo chora
Um dia a gente chega
E no outro vai embora
Cada um de nós compõe a sua história
Cada ser em si
Carrega o dom de ser capaz
E ser feliz

Almir Sater, 2011

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INTRODUCTION

In the United States, cost associated with reproductive failure in beef cattle is estimated to be in excess of $500 million/year and $480 million/year in dairy cattle, with an aggregate national total of approximately $1 billion annually (Bellows et al., 2002). In beef cows, reproductive failure can be associated with management and environment issues, cow infertility or sire infertility. A majority of the research has been focused in understanding and improving female reproductive efficiency in cattle, however evaluating male fertility is an often-sought-after endeavor for many species of domestic animals. Unfortunately, current *in vitro* methods to evaluate semen quality cannot accurately predict potential field fertility of that sample. Use of sub-fertile bulls can have a devastating impact on reproduction because one sire can breed up to hundreds of thousands of females per year using artificial insemination. Large variation in fertility still exist in semen samples that are classified as satisfactory after typical clinical and laboratory evaluations, indicating that additional assays need be included in order to test the parental contribution to pregnancy establishment and maintenance. Limited data have been reported on the paternal role on pregnancy beyond the early embryonic period, but based on the large influence that the sire plays in placental development, investigating this relationship may help elucidate some causes of reproductive failure.

CHAPTER I: NON-TRADITIONAL METHODS TO EVALUATE SIRE FERTILITY

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Abstract

Evaluation of fertility potential of a semen sample is essential to achieve good reproductive results. With increasing adoption of artificial insemination and semen cryopreservation have also increased the urge to develop methods to accurately predict sire fertility. Among sires that have satisfactory results in current fertility tests, variance of pregnancy rate still exists, and the causes remain unknown. In this review we will address three major topics. First, the most common measurements of herd fertility and the importance of using calving rate in order to get the most accurate sire fertility evaluation. Second, how to predict field fertility using in vitro approaches and the main factors that increases the uncertainty of prediction methods. Last, the role of sire in pivotal periods of gestation and its

contribution to pregnancy loss. Putting together these topics may help understand the key components in evaluating and improving sire fertility.

Introduction

For over a century, scientists have attempted to develop techniques to accurately predict the fertility potential of a male's semen sample. In most livestock species, the sire is responsible for multiple pregnancies per year and up to hundreds of thousands of pregnancies if used for artificial insemination (AI). Use of sub fertile or infertile sires can have devastating impacts in regard to the herd's reproductive efficiency. Even though fertility studies are expanding rapidly as molecular, genomic and computer techniques explode, our understanding of male fertility is still far from complete.

An ideal male fertility test must be economically practical, provide consistent results and have the ability to measure multiple variables as spermatozoa must meet many requirements for successful fertilization. A perfect test should be able to evaluate not only the ability of the spermatozoa to reach the site of fertilization, but also the ability to fertilize the oocyte, establish and maintain a successful pregnancy (Braundmeier and Miller, 2001).

How to measure fertility?

Fertility is broadly defined as the ability to produce a viable offspring. Between mating and birth, there are several time points that are used to evaluate fertility success in livestock, including fertilization rate, non-return to estrus rate,

conception rate, pregnancy rate and calving rate which are correlated with different aspects of the reproductive cycle. Even though each measurement provides useful information, emphasis of a cattle system should be placed on the final product, represented by calving rate.

Fertilization rate can be evaluated *in vitro* by presence of first cell division after 48h of insemination or *in vivo* by flushing the uterus 7 days after insemination to recover embryos or unfertilized oocytes (Sreenan and Diskin, 1986). However, the technique of flushing does not guarantee that all embryos will be recovered, limiting the use of this index as a true estimate of fertilization. This measurement overestimates the conception rate by about 10-15%, mostly due to animals in anestrus, early pregnancy loss or failure to detect estrus (Hafez and Hafez, 2013).

Conception rate represents the percentage of pregnant animals over number of animals inseminated after detection of estrus, similar to fertilization rate. However, due to limitations of early pregnancy diagnosis, conception rate is usually reported as pregnant animals at some point after breeding (e.g. 30 to 45 days) and therefore, account for not only fertilization rate, but also early pregnancy loss. Pregnancy rate is the most common reported measurement of herd fertility. It accounts for the number of animals that conceived in a defined time period over the number of animals eligible for breeding during the defined time period. This time period can be either the 21-day estrus interval, a single day of timed AI, or breeding season interval often 90 days to 120 days (DeJarnette and Amann,

2010). Identification of pregnant animals are often obtained using rectal palpation, ultrasonography or blood-based pregnancy tests.

From a productive and economic point of view, the most appropriate measurement of fertility is calving rate. Defined as the amount of calves born from total number of cows inseminated or exposed to a bull, this index accounts for both fertilization rate and all subsequent pregnancy losses. This measurement, however, provides little advantage to a producer because it can only be obtained 9 months after the breeding season.

In cattle, most male fertility traits have only been studied in relation to fertilization and early embryonic development, represented by conception or pregnancy rate. Historically, pregnancy loss after this time is usually associated with female infertility only. However, studies have shown paternal genetics provide a significant contribution to embryonic/fetal mortality in cattle (López-Gatius et al., 2002; Starbuck et al., 2004; Franco et al., in press) that can drastically affect calving rate and should be accounted for when measuring the male effect on herd fertility.

How to predict male fertility?

The relationship between *in vitro* semen quality and field fertility has been the subject of considerable study (Amann and Hammerstedt, 1993; Farrell et al., 1998; Zhang et al., 1999; Larsson and Rodrıguez-Martınez, 2000; Rodríguez-Martínez, 2003). Unfortunately, most current methods cannot accurately describe the fertility level that the analyzed semen will reach *in vivo*. Instead these estimates

are only established with field fertility tests after several hundreds or thousands of females have been inseminated. This can cost money and time for both semen companies and producers. For this reason, analyses of fresh and processed semen have become more detailed, moving from simply evaluating sperm traits (e.g. motility, morphology, structure integrity) to determining fertilizing potential of the sample through genetic and molecular markers and aiming for prediction of prognostic values (Rodríguez-Martínez, 2003).

There are several factors that affect semen quality and increase difficulty of estimating fertility; including variability between ejaculates, processing batches, environmental factors and semen handling after leaving the bull stud. Health, reproductive status and management practices of bulls and cow herd during breeding season also contributes significantly for variability in fertility (Figure 1). A review presented by (Utt, 2016) pointed out that in order to maximize accuracy and precision of fertility prediction, relevant sources of variation should be accounted for. Unexplained variation and the number of observations, create a degree of uncertainty, or “noise” that compromises the precision of sire fertility prediction. If differences in fertility predictions among bulls are not greater than the “noise”, the likelihood of accurately predicting the difference on fertility among bulls is minimal.

How to improve sire fertility?

Variation in pregnancy rate to TAI caused by sire fertility is substantial. Understanding factors that affect the establishment and maintenance of pregnancy

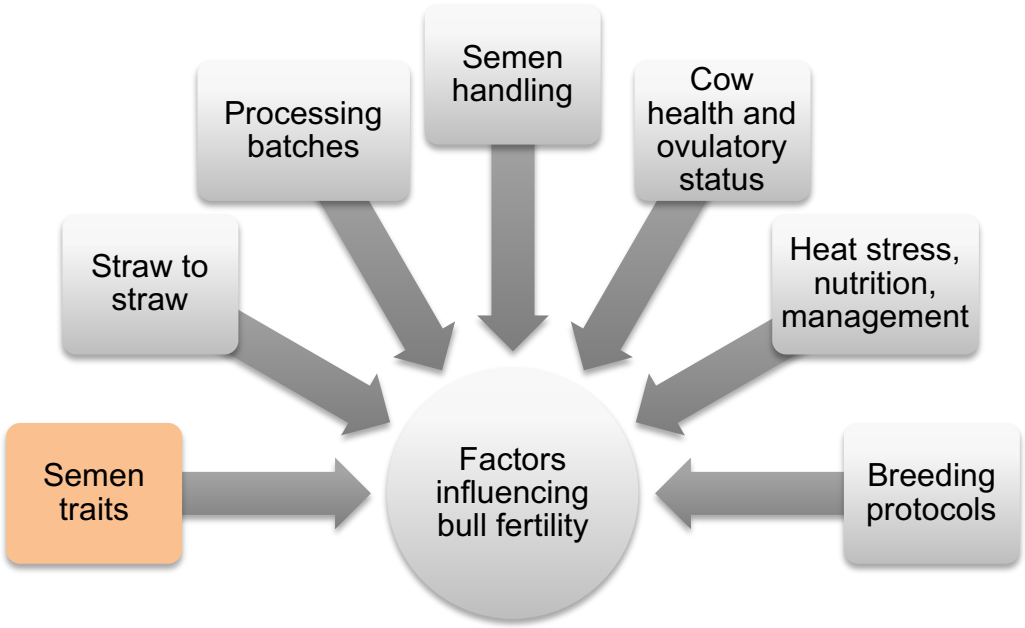

Figure 1. Factors influencing bull fertility prediction.

Variables that can affect prediction of bull fertility includes variability related to the semen itself, including inherently variability of the measure (semen traits), straw-to-straw, processing batches and the semen handling after leaving the stud, as well as variance related to management and female components. Adapted from Utt (2016).

is critical to developing management strategies to increase fertility. Reproductive performance g radually improves as sperm number per ejaculate increases, until a plateau is achieved above which additional increases in sperm number or quality does not improve fertility (DeJarnette and Amann, 2010). The physiological basis for this response is based on the concept that a threshold number of competent sperm need to be transported to the oviduct to successfully fertilize the ova. Hence, improvements in fertility will occur with additional sperm until this threshold level is reached. However, a greater insemination dose beyond this point does not enhance fertility because the optimal population needed for successful fertilization is already present in the oviduct (Salisbury and Vandemark, 1961). This concept is based on the presence of compensable or noncompensable traits in the sperm. Compensable traits are those that do not affect fertility, if high numbers of spermatozoa are used during insemination and include motility, morphology, ability to undergo capacitation and initiate an acrosome reaction. In contrast, noncompensable traits are those that cannot be overcome by increasing the number of spermatozoa in the insemination dose (Saacke et al., 2000; Braundmeier and Miller, 2001). These defects affect the function of spermatozoa during the later stages of fertilization and embryonic development, such as nuclear vacuoles (Saacke et al., 1988), morphological deficiencies that do not suppress movement (DeJarnette et al., 1992), and defective chromatin structure (Ballachey et al., 1988). Understanding the form and function of spermatozoa and

consequences of particular defects is imperative to developing methods to identify high fertility sires and improve reproductive efficiency.

Sire contribution to fertilization

To achieve fertilization, sperm have to swim from site of deposition to the ampulla of the oviduct, during this process undergo capacitation to be able to bind to zona pellucida and successfully fertilize the oocyte. The female reproductive tract is the first physical barrier to sperm transport, and good sperm concentration and motility is crucial for efficient sperm transport. In natural breeding, the number of sperm deposited in female tract is usually far above the required, therefore concentration is not a problem. In the case of frozen semen, AI organizations sell straws that contain a number of sperm anticipated to provide optimum probability of fertilization, based on historic data for bull in general, and specific semen quality and fertility data for individual bulls. (DeJarnette and Amann, 2010). Thus insufficient number of spermatozoa's reaching the oocyte is not likely a major cause of reproductive failure. The female reproductive tract interacts with sperm in order to facilitate transport to the site of fertilization at the same time as selecting the best sperm for fertilization and impeding entrance of pathogens into the tract. Recent research has been done in order to understand the communication between sperm and epithelium and the effect of that in sperm competence and fertilization success (Suarez, 2016). Studies in mice have shown that motility alone does not enable sperm to pass through the uterotubal junction, and the presence of certain proteins on sperm plasma membrane somehow interacts with the lining

of uterotubal junction and might be necessary for sperm oocyte interaction. (Okabe, 2015).

When sperm reaches the oviduct, they bind to epithelium, forming a storage reservoir until ovulation. This process seems to maintain sperm viability as well as prevent polyspermia by allowing only a few sperm at a time to reach oocytes (Pollard et al., 1991; Chian and Sirard, 1995). In cattle, Binder of Sperm 1 [BSP - 1] is the most abundant protein in bovine seminal plasma, and it has been shown to prolong sperm viability by acting to stabilize the plasma membrane during this storage period (Greube et al., 2001). During the process of sperm capacitation, specific changes need to happen in order to promote the detachment of sperm from oviductal epithelium to meet and bind to the oocyte. These modifications are not well established, but might be related with modification of sperm surface proteins that could reduce binding affinity for oviductal receptors or with sperm hyperactivation, that could provide the force necessary for sperm to pull away from the oviductal epithelium (Suarez, 2016). Capacitated bull sperm showed reduced binding to oviductal epithelium (Lefebvre and Suarez 1996), which might be attributable to loss or modification of the BSP proteins on the sperm surface. In summary, no single factor, male or female derived, likely dictates the ultimate success or failure of sperm to achieve fertilization, but in fact, it is obvious that several factors have a role in this process.

Sire contribution to early pregnancy

It is well established that both the oocyte and sperm contribute to the embryonic genome. Several studies have shown that disruptions in paternal DNA negatively impact embryo quality (Virro et al., 2004; Benchaib et al., 2007; Simon and Lewis, 2011). Poor sperm quality can delay pronuclear formation and therefore embryonic development, due to reduced DNA integrity. However, there is much debate in the literature about the extension of maternal and paternal controls of early stages of embryo development. In humans it has been shown that sperm damaged chromatin influence all embryo development stages, however, the effect is more prominent after embryonic genome activation (Simon and Lewis, 2011). Other studies demonstrate that irreparable abnormalities in paternal genome affect blastocyst development even when intracytoplasmic sperm injection is used to fertilize oocytes (Jones et al., 1998). Therefore, evaluating chromatin and DNA integrity, together with others semen quality tests may be a valid approach to identify and diagnosis normal male fertility. Sperm chromatin structure assay [SCSA] is a technique to measure the percentage of sperm with a high susceptibility to low pH- induced DNA denaturation and is expressed as the DNA fragmentation index (Virro et al., 2004). In cattle, SCSA data was significantly correlated with bull stud field fertility (Ballachey et al., 1987) data and bull fertility measured by heterospermic performance (Ballachey et al., 1988).

Semen cryopreservation and artificial insemination has been a major advance in the livestock industry, however, the process of cooling, freezing and

thawing can damage the sperm membrane structures, and consequently reduce semen quality when compared with fresh semen. The composition of extender used during the process of semen preservation can also alter semen chromatin quality (Karabinus et al., 1991). The effect of sperm DNA damage in cattle reproduction has not been well documented as in human assisted reproductive technologies, but it is expected to be similar. Sperm DNA integrity is a non-compensable trait, that means, it cannot be compensate by increasing sperm dose concentration. Therefore, further research to develop methods to reduce the risk of DNA damage during semen preservation as well as identifying bulls with high incidence of genetic disorders can help improve male fertility.

Sire contribution to late pregnancy

Previously, the impact of poor quality spermatozoa was restricted to early pregnancy loss and most subsequent reproductive failure was attributed to a female deficit, environmental factors, or lethal defects in the embryo itself. However, many field fertility studies have shown differences between bulls' ability to sire successful pregnancies that go to term and led to a live offspring (Markusfeld-Nir, 1997; López-Gatius et al., 2002; Pegorer et al., 2007; Franco et al., in press). Based on the significant influence that sire plays in placental development, evaluating the relationships between sire and pregnancy loss could provide valuable insight to male fertility. In embryos derived from only maternal genetics, the embryo proper (i.e. body, organs, etc.) develops, but placental development is dramatically limited resulting in death of the embryo. Alternatively,

embryos derived from only paternal or male genetics results in no embryo proper but a robust placenta (Surani et al., 1987b, a). Recently, Selvaraju et al. (2017) reported the presence of placental development-associated transcripts, such as *PAG5*, *PAG7* and *PAG10* in full length and intact spermatozoa, suggesting that sperm-borne transcripts potentially have an influence beyond early embryonic development, such as during the processes of implantation and placentation.

Conclusion

Prediction of male fertility is a constant effort in both the beef and dairy industry worldwide. Developing *in vitro* techniques to accurately predict field fertility would have a major impact on increasing overall reproductive efficiency. In this review we pointed out important aspects to be considered when evaluating the effect of sire on pregnancy establishment and maintenance. It is important to realize that even when sperm appear to possess all the required traits for successful fertilization, relative difference still exist, emphasizing the need for a better understanding of sperm molecular and genetic characteristics as well as how spermatozoa interact with the female reproductive tract after insemination. Another key point is that sires have a significant contribution to pregnancy loss, that should not to be ignored when measuring fertility. Exploring this relationship can help understanding the causes of pregnancy loss, as well as developing tools to identify and select higher fertility sires.

References

Amann, R., and R. Hammerstedt. 1993. In vitro evaluation of sperm quality: an opinion. Journal of Andrology 14: 397-406.

Ballachey, B., W. Hohenboken, and D. Evenson. 1987. Heterogeneity of sperm nuclear chromatin structure and its relationship to bull fertility. Biology of reproduction 36: 915-925.

Ballachey, B. E., D. P. Evenson, and R. G. SAACKE. 1988. The sperm chromatin structure assay relationship with alternate tests of semen quality and heterospermic performance of bulls. Journal of andrology 9: 109-115.

Bellows, D., S. Ott, and R. Bellows. 2002. Review: Cost of Reproductive Diseases and Conditions in Cattle. The Professional Animal Scientist 18: 26-32.

Benchaib, M. et al. 2007. Sperm deoxyribonucleic acid fragmentation as a prognostic indicator of assisted reproductive technology outcome. Fertility and sterility 87: 93-100.

Braundmeier, A., and D. Miller. 2001. Invited review: the search is on: finding accurate molecular markers of male fertility. Journal of dairy science 84: 1915-1925.

Chian, R.-C., and M.-A. Sirard. 1995. Fertilizing ability of bovine spermatozoa cocultured with oviduct epithelial cells. Biology of reproduction 52: 156-162.

DeJarnette, J., and R. Amann. 2010. Understanding estimates of AI sire fertility: From A to Z. In: 23rd technical conference on artificial insemination & reproduction, Milwaukee, WI

DeJarnette, J., R. Saacke, J. Bame, and C. Vogler. 1992. Accessory sperm: their importance to fertility and embryo quality, and attempts to alter their numbers in artificially inseminated cattle. Journal of animal science 70: 484-491.

Farrell, P., G. Presicce, C. Brockett, and R. Foote. 1998. Quantification of bull sperm characteristics measured by computer-assisted sperm analysis (CASA) and the relationship to fertility. Theriogenology 49: 871-879.

Franco, G. A. et al. in press. Sire contribution to pregnancy loss and pregnancy associated glycoproteins (PAGs) production in Nelore cows. Journal of Animal Science.

Greube, A., K. Müller, E. Töpfer-Petersen, A. Herrmann, and P. Müller. 2001. Influence of the bovine seminal plasma protein PDC-109 on the physical state of membranes. Biochemistry 40: 8326-8334.

Hafez, E. S. E., and B. Hafez. 2013. Reproduction in farm animals. John Wiley & Sons.

Jones, G. M., A. O. Trounson, N. Lolatgis, and C. Wood. 1998. Factors affecting the success of human blastocyst development and pregnancy following in vitro fertilization and embryo transfer 1. Fertility and Sterility 70: 1022-1029.

Karabinus, D., D. Evenson, and M. Kaproth. 1991. Effects of egg yolk-citrate and milk extenders on chromatin structure and viability of cryopreserved bull sperm. Journal of dairy science 74: 3836-3848.

Larsson, B., and H. Rodrıguez-Martınez. 2000. Can we use in vitro fertilization tests to predict semen fertility? Animal Reproduction Science 60: 327-336.

López-Gatius, F., P. Santolaria, J. Yaniz, J. Rutllant, and M. López-Béjar. 2002. Factors affecting pregnancy loss from gestation day 38 to 90 in lactating dairy cows from a single herd. Theriogenology 57: 1251-1261.

Markusfeld-Nir, O. 1997. Epidemiology of bovine abortions in Israeli dairy herds. Preventive Veterinary Medicine 31: 245-255.

Mercadante, P. M. et al. 2013. Subspecies differences in early fetal development and plasma pregnancy-associated glycoprotein concentrations in cattle1. Journal of Animal Science 91: 3693-3701.

Okabe, M. 2015. Mechanisms of fertilization elucidated by gene-manipulated animals. Asian journal of andrology 17: 646.

Pegorer, M. F., J. L. M. Vasconcelos, L. A. Trinca, P. J. Hansen, and C. M. Barros. 2007. Influence of sire and sire breed (Gyr versus Holstein) on establishment of pregnancy and embryonic loss in lactating Holstein cows during summer heat stress. Theriogenology 67: 692-697.

Pollard, J. W. et al. 1991. Fertilizing capacity of bovine sperm may be maintained by binding to oviductal epithelial cells. Biology of reproduction 44: 102-107.

Rodríguez-Martínez, H. 2003. Laboratory semen assessment and prediction of fertility: still utopia? Reproduction in Domestic Animals 38: 312-318.

Saacke, R., J. Bame, D. Karabinus, J. Mulins, and S. Whitman. 1988. Transport of abnormal sperm in the artificially inseminated cow based upon accessory

sperm in the zona pellucida. In: Proceedings of the 11th International Congress on Animal Reproduction and Artificial Insemination. p 292-294.

Saacke, R., J. Dalton, S. Nadir, R. Nebel, and J. Bame. 2000. Relationship of seminal traits and insemination time to fertilization rate and embryo quality. Animal reproduction science 60: 663-677.

Salisbury, G. W., and N. L. Vandemark. 1961. Physiology of reproduction and artificial insemination of cattle. Physiology of reproduction and artificial insemination of cattle.

Selvaraju, S. et al. 2017. Occurrence and functional significance of the transcriptome in bovine (Bos taurus) spermatozoa. Sci Rep 7.

Simon, L., and S. E. Lewis. 2011. Sperm DNA damage or progressive motility: which one is the better predictor of fertilization in vitro? Systems biology in reproductive medicine 57: 133-138.

Sreenan, J., and M. Diskin. 1986. The extent and timing of embryonic mortality in the cow Embryonic mortality in farm animals. p 1-11. Springer.

Starbuck, M. J., R. A. Dailey, and E. K. Inskeep. 2004. Factors affecting retention of early pregnancy in dairy cattle. Anim Reprod Sci 84: 27-39.

Suarez, S. S. 2016. Mammalian sperm interactions with the female reproductive tract. Cell and tissue research 363: 185-194.

Surani, M. A., S. C. Barton, and M. L. Norris. 1987a. Experimental reconstruction of mouse eggs and embryos: an analysis of mammalian development. Biol Reprod 36: 1-16.

Surani, M. A., S. C. Barton, and M. L. Norris. 1987b. Influence of parental chromosomes on spatial specificity in androgenetic----parthenogenetic chimaeras in the mouse. Nature 326: 395-397.

Utt, M. D. 2016. Prediction of bull fertility. Animal Reproduction Science 169: 37-44.

Virro, M. R., K. L. Larson-Cook, and D. P. Evenson. 2004. Sperm chromatin structure assay (SCSA®) parameters are related to fertilization, blastocyst development, and ongoing pregnancy in in vitro fertilization and intracytoplasmic sperm injection cycles. Fertility and sterility 81: 1289-1295.

Zhang, B., B. Larsson, N. Lundeheim, M. Haard, and H. Rodriguez-Martinez. 1999. Prediction of bull fertility by combined in vitro assessments of frozen-thawed semen from young dairy bulls entering an AI-programme. International journal of andrology 22: 253-260.

CHAPTER II: SIRE CONTRIBUTION TO PREGNANCY LOSS AND PREGNANCY ASSOCIATED GLYCOPROTEIN [PAG] PRODUCTION IN NELORE COWS

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Abstract

Pregnancy loss is a major contributing factor to reproductive inefficiency in both the beef and dairy industries. Sires can have a significant influence on the amount of pregnancy loss; however, this relationship is still poorly investigated. The primary objective of this study was to identify sires associated with high or low incidence of pregnancy loss (between d 30 and 100 of gestation) and investigate their effect on concentration of circulating pregnancy associated glycoproteins [PAGs]. Postpartum multiparous Nelore cows were inseminated artificially at a fixed time [FTAI, d 0] after synchronization of ovulation. A total of 736 cows were assigned randomly to be inseminated with semen from either of 6 Angus sires, whereas a separate subset of 492 cows were inseminated randomly with semen from either of 3 Nelore (*n* = 235) or either of 2 Angus sires (*n* = 257). Estrus expression was evaluated on d 0 using Estrotect Heat Detector patches. Blood

samples were collected on d 30 of gestation for quantification of PAGs and pregnancy diagnosis was performed by transrectal ultrasound on d 30 and 100 after FTAI. Cows diagnosed pregnant at the first examination but not pregnant at the second were defined to have pregnancy loss. Overall pregnancy rate at d 30 was 54% (660/1228) and pregnancy loss was 6.21% (41/660). Cows receiving semen from Nelore sires had greater ($P < 0.001$) pregnancy rate, greater ($P = 0.014$) pregnancy loss and lesser ($P = 0.002$) PAG concentrations at d 30 of gestation compared with cows receiving Angus semen. Circulating PAG concentrations were lower ($P = 0.008$) in cows that had pregnancy loss (9.76 ± 0.25 vs 7.41 ± 1.02 ng/ml). Angus sires were retrospectively classified according to percentage of pregnancy loss as either high pregnancy loss (mean of 7.25% or 67% of total) or low pregnancy loss (mean of 3.93% or 33% of total). Cows receiving semen from high pregnancy loss sires had 1.9 times greater *(P* = 0.123*)* rate of pregnancy loss than cows mated to low pregnancy loss sires and had lower ($P = 0.059$) PAG concentrations at d 30 of gestation. In summary, PAG concentrations reflected probability of pregnancy maintenance and were influenced by both sire and sire breed used at FTAI. A large variation in the incidence of pregnancy loss was detected among sires that could not be predicted with standard semen fertility evaluations. Exploring the relationship of sire and PAG production might be promising to improve sire selection with regard to pregnancy loss.

Introduction

Pregnancy loss (between d 30 and 100 of gestation) contributes to significant adverse impacts on production and economic efficiency in cattle systems. Even though all mechanisms causing late embryonic/early fetal mortality are unknown, it may be related to insufficient placental development. Bovine pregnancy-associated glycoproteins [PAGs] are secreted from binucleate trophoblast cells of the placenta and have been used to monitor embryonic or fetal viability as well as placental function in cattle (Perry et al., 2005; Thompson et al., 2010; Pohler et al., 2013; Pohler et al., 2016a). Cows having pregnancy loss during the late embryonic/early fetal period had significantly reduced PAG concentrations compared with cows that maintained pregnancy (Pohler et al., 2016b). Limited information has been reported on the influence of sire on circulating PAGs, but based on the large influence that the sire has on placental formation, there is considerable interest in this relationship. Different studies have shown that parthenogenomes (Kaufman et al., 1977; Surani et al., 1987) and gynogenones (Surani et al., 1984; Barton et al., 1985), embryos with only a maternal genome, develop with poor placental proliferation, but a reasonably well-developed embryo proper, whereas androgenones, embryos derived from only a paternal genome, result in no embryo proper development, but form a robust placenta (McGrath and Solter, 1984; Surani et al., 1984; Barton et al., 1985). In addition, substantial variation in the amount of pregnancy loss exists between sires with comparable initial pregnancy rate (López-Gatius et al., 2002; Starbuck et al., 2004). Therefore,

the objectives of these experiments were to identify sires associated with high or low pregnancy loss and to investigate their effect on circulating concentrations of PAG. We hypothesized that pregnancies sired by high pregnancy loss sires will have decreased circulating PAGs at d 30 of gestation compared with pregnancies sired by low pregnancy loss sires.

Materials and Methods

This study was conducted on a commercial beef farm in Mato Grosso, Brazil following the recommendations of the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 1999). This study included a total of 1,228 suckled multiparous Nelore cows (average BCS = 3.24), separated in 10 management groups with approximately 120 cows in each, maintained on pastures of *Brachiaria brizantha* with water and mineral salt ad libitum. All cows were at least 20 d postpartum (average 51 d; ranging from 22 to 79 d) when subjected to the following estrus-synchronization protocol: an intravaginal progesterone (P4) insert containing 1.9 g of P4 (CIDR; Zoetis, São Paulo, Brazil), and 2.0 mg (im) estradiol benzoate (2.0 mL of Gonadiol; Zoetis, São Paulo, Brazil) on d -11, CIDR withdrawal, 12.5 mg (im) dinoprost tromethamine (PGF; 2.5 mL of Lutalyse; Zoetis, Brazil), 300 IU of equine chronic gonadotropin (1.5 mL of Novormon; Zoetis, São Paulo, Brazil), and 0.6 mg (im) of estradiol cypionate (0.3 mL of E.C.P.; Zoetis, Brazil) on d -2, and fixed-time artificial insemination [FTAI] on d 0 (Meneghetti et al., 2009). Estrotect Heat Detection patches were placed at d -2 and scored at the time of AI on a scale of 0 to 4 (0, lost patch; 1, <25%

activated; 2, <50% activated; 3, <75% activated; and 4, >75% activated) as described by Pohler et al. (2016b). Cows with patch scores 1 and 2 were defined to not be in estrus, whereas patch scores of 3 and 4 signified estrus had occurred. Cows with patch scores of 0 were removed from the analysis that evaluated estrus expression.

After FTAI, all cows underwent pregnancy diagnosis at d 30 and 100 of gestation by transrectal ultrasonography (Aloka 500V, Aloka, Wallingford, CT) with a 7.5 MHz transrectal linear probe. Positive pregnancy status was based on the presence of a viable embryo with a heartbeat. Cows diagnosed pregnant at the first examination (d 30) but non-pregnant at d 100 were defined to have pregnancy loss.

Blood Sampling

Blood samples were collected from cows diagnosed pregnant at d 30 post FTAI by tail venipuncture into a 10-mL vacutainer tube (BD Vacutainer, Becton, Dickinson and Company, New Jersey) and allowed to clot at room temperature for 1 pregnancy loss before being placed in a 4°C refrigerator for approximately 24 pregnancy loss . Samples were centrifuged at 1,500 x g for 15 min and stored at -20 °C until measurement of PAGs.

Sire Distribution

A majority of cows (6 management groups, n = 736) were assigned randomly to be inseminated with semen from either of 6 Angus sires, whereas another subset of cows (4 management groups, n = 492) were inseminated

randomly with either semen from either of 3 Nelore (n = 235) or either of 2 Angus sires (n = 257) to assess the effects of sire breed on incidence of pregnancy loss and d 30 PAG concentrations. To ensure randomization, sires were alternated every 10 cows and 4 different sires were used in each management group. Mean number of inseminations per sire was 122 for first subset and 98 for second subset, ranging from 53 to 131. Straws of frozen semen were purchased from major semen companies that follow CSS (Certified Semen Services) and all health, ethical and animal welfare NAAB (National Association of Animal Breeders) guidelines. All semen passed pre-freezing quality tests with a minimum of 30% progressive motility and 70% normal morphology as well as post thaw testing at each company before reaching the farm.

Assay Procedure

Serum concentration of PAGs was determined by a monoclonal-based PAG ELISA similar to that described by Green et al. (2005) using a polyclonal antibody (Ab 63) as described by Reese et al. (2017) to quantify PAGs secreted early in gestation, with a sensitivity of 0.28 ng/mL. Each assay was run with duplicates of each sample, a standard curve, a sample from a pregnant cow approximately 60 d in gestation, and a pooled sample from non-pregnant cows as controls. Intra and inter assay coefficients of variation were less than 10%.

Statistical Analyses

One-way ANOVA (GLM procedure, SAS 9.4, Institute Inc., Cary, NC) was used to test differences in the dependent variables pregnancy rate, pregnancy loss

and PAG concentrations. For pregnancy rate and pregnancy loss, fixed effects included sire breed (Angus or Nelore), sire fertility (high or low pregnancy loss) and estrus expression (yes or no). For PAG concentrations, fixed effects included pregnancy status (maintenance or loss), sire breed, sire fertility. Cow BCS, age, days postpartum, management group, semen collection number and PAG plate were included as random variable in all models. All data were analyzed using cow as the experimental unit and means were separated using LSMEANS and adjusted according to the Tukey-Kramer test. Frequency of pregnancy rate and pregnancy loss was compared between variables using odds ratio (FREQ procedure, SAS 9.4, Institute Inc., Cary, NC). Probability for prediction of pregnancy maintenance by circulating PAGs concentration was determined according to the following equation: Probability = ($e^{\text{logistic equation}}$)/(1 + $e^{\text{logistic equation}}$) using GENMOD procedure (SAS 9.4, Institute Inc., Cary, NC). For all analyses, significance was set at $P \leq 0.05$ and tendencies were determined when $0.05 < P \leq 0.15$, results are presented as mean ± SEM.

Results

Overall pregnancy rate following FTAI at d 30 was 54% (660/1228) and pregnancy loss between d 30 and 100 was 6.21% (41/660). Cows inseminated with semen from Angus sires had decreased ($P < 0.001$) pregnancy rate at d 30 (50.95 ± 1.57 vs 65.53 ± 3.23%) and decreased ($P = 0.014$) incidence of pregnancy loss (4.94 ± 0.09 vs 10.39 ± 2.46, %) compared with cows inseminated with semen from Nelore sires. In addition, the breed of sire had a significant effect on circulating

concentrations of PAG at d 30 of gestation. Pregnant Nelore cows inseminated with Angus semen had greater (P = 0.002) serum concentration of PAGs on d 30 compared with cows with Nelore sired pregnancies (11.99 ± 0.53 vs 9.73 ± 0.49 ng/ml; Fig. 2). Multiparous Nelore cows that had a viable pregnancy on d 30, but diagnosed non-pregnant on d 100 of gestation were classified as having pregnancy loss and had decreased (P = 0.008) circulating concentration of PAGs on d 30 compared with cows that maintained pregnancy until d 100 (9.76 ± 0.25 vs 7.41 ± 1.02 ng/ml; Fig. 3). For each 1 ng/mL increase in circulating concentration of PAGs at d 30, the odds of pregnancy maintenance to d 100 of gestation, increased (P = 0.006) by 11%.

Pregnancy rate at d 30 did not differ among Angus sires (P > 0.05; range 49-55%), except sire AN6 which had a lower (P = 0.006) d 30 pregnancy rate (35%); however, no clear relationship was detected between pregnancy rate at d 30 or 100 post FTAI and circulating concentration of PAGs at d 30 (Fig. 4). Angus sires used at FTAI were retrospectively classified according to percentage of pregnancy loss between d 30 and 100 of gestation as high pregnancy loss (3 sires with the greatest pregnancy loss, 7.25% mean) or low pregnancy loss (3 sires with the least pregnancy loss, 3.93% mean; Table 1). High pregnancy loss sires accounted for 66% of the total pregnancy loss and cows mated to these sires had 1.9 times greater rate of pregnancy loss than cows mated with low pregnancy loss sires (P = 0.123). After removing data from cows that had pregnancy loss, circulating PAG concentrations in pregnancies sired by the high pregnancy loss

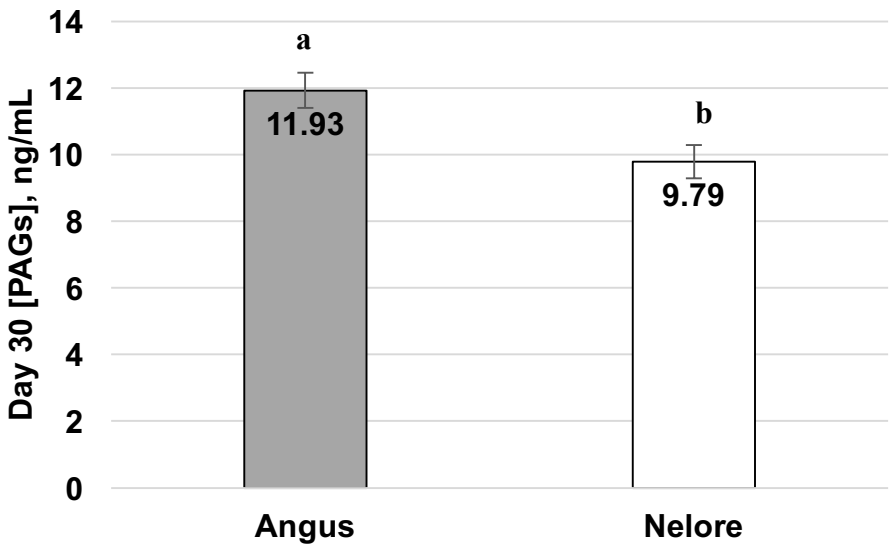

Figure 2: Effect of sire breed on circulating PAGs concentration.

Serum concentrations of PAG (mean ± SEM) in pregnant cows that were FTAI with Angus (*n* = 131) or Nelore (*n* = 138) semen and had a viable pregnancy up to d 100 of gestation. Viable pregnancies by Angus sires had greater PAGs concentration (*P* = 0.003) compared with Nelore pregnancies. a, b denotes a significant difference.

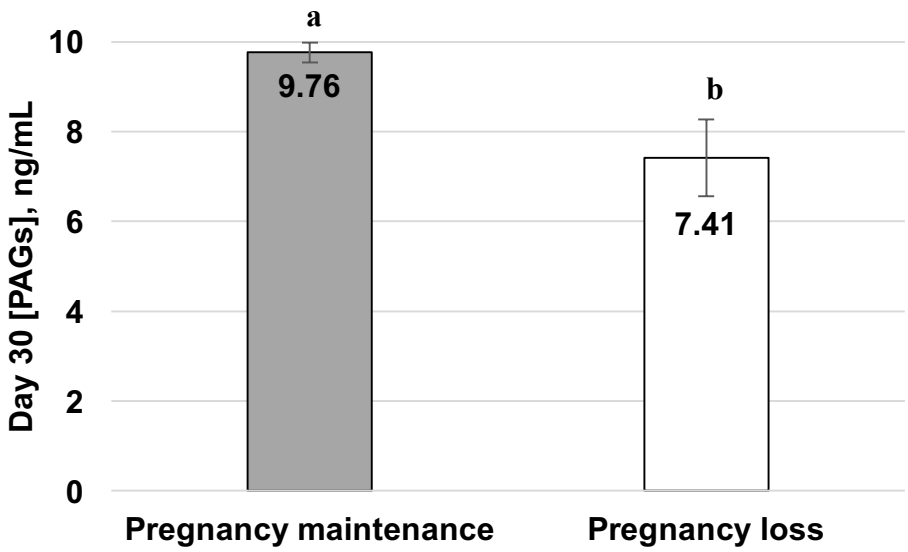

Figure 3: Circulating PAGs concentration by pregnancy success.

Serum concentrations of PAG (mean ± SEM) in cows that had a viable embryo on d 30 of gestation (*n* = 660) and either maintained (pregnancy maintenance; *n* = 619) or experienced pregnancy loss (pregnancy loss; *n* = 41). Cows that experienced pregnancy loss by d 100 of gestation had decreased (*P* = 0. 0.008) circulating concentrations of PAGs on d 30 compared with cows that maintained pregnancy until d 100. a, b denotes a significant difference.

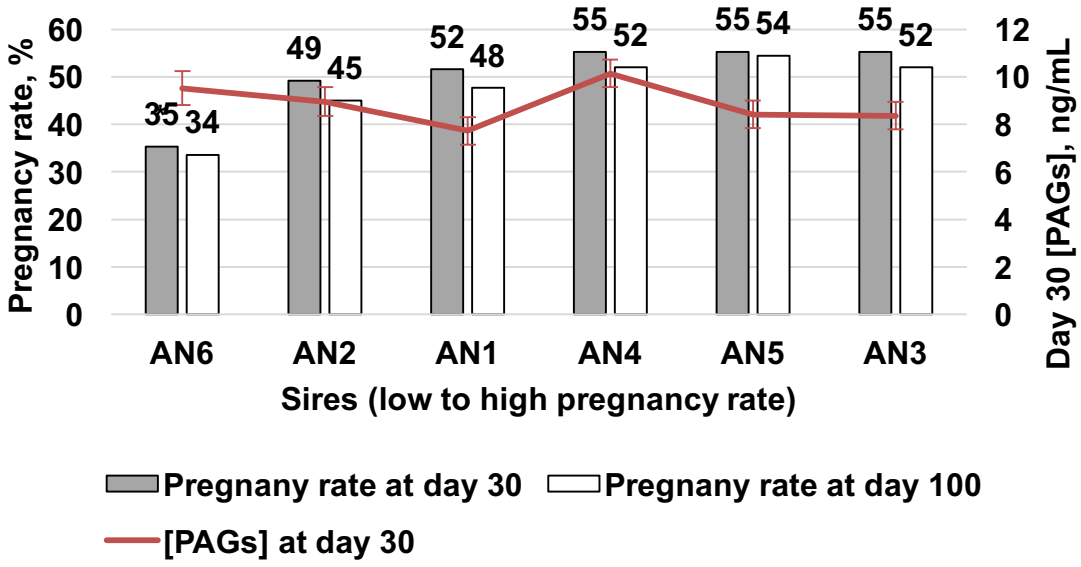

Figure 4: Relationship between circulating PAG concentrations and pregnancy rate at d 30 and 100 after FTAI by sire.

Six Angus (AN) sires are shown in order of low to high pregnancy rate (left to right). Sire AN6 was the only sire with lower (*P* = 0.006) pregnancy rate at d 30 and 100. Circulating PAG concentrations from viable pregnancies from each sire are represented in secondary axis (mean ± SEM). * denotes a significant difference.

Table 1. Classification of Angus sire fertility according to percentage of pregnancy loss (*n* = 371 pregnancies).

Pregnancy loss classification	Sires	Mean pregnancy loss (% of pregnancies)	% of total
High	AN1, AN2, AN3	7.25 (n = 193)	66.67
Low	AN4, AN5, AN6	3.93 (n = 178)	33.33

sires decrease (P = 0.059) on d 30 of gestation compared with pregnancies sired by low pregnancy loss sires (8.53 ± 0.35 vs 9.49 ± 0.36 ng/ml; Fig. 5).

Estrus expression before FTAI influenced reproductive results. In general, cows that expressed estrus had greater (P < 0.001) pregnancy rate at d 30 (64.84 vs 43.25%) compared with those that did not express estrus. Odds ratio test revealed that cows which expressed estrus had 2.7 times greater (P < 0.001) odds of being pregnant at d 30 and 2.6 times greater (P < 0.001) odds of being pregnant up to d 100 compared with those that did not express estrus. The percentage increase in pregnancy rate when cows expressed estrus was highly variable between sires (4 to 51%, Table 2), even though the percentage of cows expressing estrus was not different (P = 0.413) among sires (range 44 to 68%). Pregnancy rate at d 30 from five of the six Angus sires was lower when cows did not express estrus at the time of FTAI compared with cows that exhibited estrus. Estrus expression did not have an influence on pregnancy rate as strongly in cows inseminated with semen from to Nelore sires compared with cows inseminated with Angus semen (Table 2). Overall, the incidence of pregnancy loss (cows diagnosed pregnant on d 30 and non-pregnant on d 100) was similar (5.77 vs 7.20%, P = 0.475) when cows expressed estrus (25/438) compared with no estrus expression before FTAI (16/222).

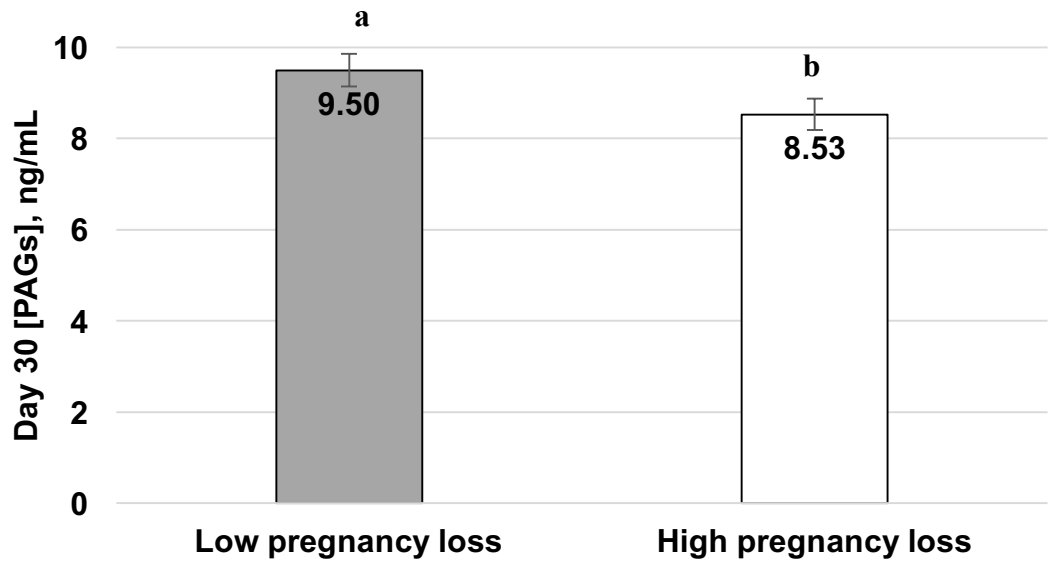

Figure 5: Circulating PAGs concentration by sire fertility.

Serum concentrations of PAG (mean ± SEM) in cows inseminated with semen from one of the six Angus sires (*n* = 371) that had a viable pregnancy up to d 100 of gestation. Three sires accounted for 66.7% of the pregnancy loss and were classified as high pregnancy loss and their viable pregnancies (after removing data from pregnancy loss) had lower circulating concentrations of PAG on d 30 (*P* = 0.059) compared with the other three sires classified as low pregnancy loss. a, b denotes a significant difference.

Table 2. Effect of estrus expression before FTAI on pregnancy rate at d 30 by sire.

Sire[1]	Pregnancy rate at d 30, %		*P*-value
	Estrus (n = 679)	No estrus (n = 549)	
AN1	60.46±5.14	33.33±7.36	0.0026
AN2	62.50±4.09	33.04±4.44	<0.0001
AN3	72.59±4.10	35.96±4.46	<0.0001
AN4	64.93±5.43	39.13±7.03	0.0038
AN5	65.33±5.50	39.58±6.88	0.0036
AN6	40.00±6.15	30.50±6.21	0.2782
NEL7	78.26±9.94	60.00±8.71	0.1676
NEL8	61.11±7.95	58.62±8.86	0.8343
NEL9	78.43±6.68	59.09±5.87	0.0299

[1] AN represents Angus sires and NEL represents Nelore sires.

Discussion

Bovine PAGs are products of binucleate trophoblast cells in the bovine placenta and can be detected in the maternal circulation beginning 24 to 26 d after insemination (Green et al., 2005; Pohler et al., 2013; Reese et al., 2017). Even though their physiological role and mechanism of potential action remain unclear, it has been hypothesized that PAGs may act in camouflaging fetal or placental antigens from the maternal immune system, process growth factors, or facilitate adhesion actions at the fetal maternal interface (Wallace et al., 2015). It has been well established that circulating PAG concentrations are an effective method of pregnancy diagnosis in cattle and sheep using either blood or milk samples (Zoli et al., 1992; Green et al., 2005; Karen et al., 2015; Wallace et al., 2015; Reese et al., 2017). Moreover, multiple reports have associated concentration of PAGs with probability of embryonic mortality or pregnancy maintenance in cattle (Perry et al., 2005; Breukelman et al., 2012; Pohler et al., 2013; Pohler et al., 2014; Pohler et al., 2016a; Pohler et al., 2016b). In the present study, similar results were observed in which circulating concentration of PAGs were decreased in cows that had pregnancy loss between d 30 and 100 of gestation. For each 1 ng/mL increase in circulating PAG concentration at d 30, the odds of pregnancy maintenance up to d 100 of gestation increased by 11%. An additional finding was that with a newly validated PAG antibody (Ab 63; Reese et al., 2017), we were able to predict late embryonic mortality, which has previously been reported to be antibody and PAG specific (Gatea et al., 2017).

The mechanism behind late embryonic/early fetal loss has not been well established, but it has been suggested to be related to a deficiency in placentation around the time of embryo attachment to the uterus (d 21 through 42 of gestation; Stice et al., 1996; Hill et al., 2000). Using PAGs as a marker of placental function and pregnancy maintenance, we started investigating the possible cause of late embryonic/early fetal loss in cattle by exploring the factors that significantly affect circulating PAGs. Initially, it was hypothesized that PAG production was driven by embryo development itself, where greater circulating concentrations of PAGs would be correlated to a more robust or larger embryo and decreased PAG concentrations would correlate to a smaller embryo. In contrast, Pohler et al. (2014) showed that there was no correlation between crown rump length, embryonic width, or embryonic volume at d 35 or 56 of gestation and d 35 or 56 circulating PAGs, indicating that decreased PAGs concentration is not merely a result of a smaller, potentially developmentally delayed embryo. Furthermore, maternal factors do not seem to be the sole driver of circulating PAGs because no repeatability was detected in circulating PAG concentrations between successive pregnancies in the same maternal environment (Reese et al., 2016). These studies together indicate that other factors play a role in PAG secretion during a single pregnancy.

The primary objective of this study was to explore the paternal contribution to pregnancy loss and PAG secretion. Our interest in this potential relationship stems from the large influence paternal genetics have on placenta formation

(Barton et al., 1985; Surani et al., 1987), and from previous studies showing differences between bulls' ability to sire successful pregnancies (Markusfeld-Nir, 1997; López-Gatius et al., 2002; Pegorer et al., 2007). Even when bulls are specifically selected for AI and pass all standard semen evaluations, significant differences exist in the sire's ability to generate and maintain successful pregnancies that cannot be explained by variation in visual semen analysis. Sires used in this experiment passed standard commercial semen analysis and meet all minimum requirements for use in the field. From the subset of cows inseminated with Angus semen, pregnancy rate at d 30 did not differ among sires, with the exception of sire AN6 which was significantly lower than the others. Even without much variation in initial pregnancy rate at d 30 post AI, there was a large variation in pregnancy loss between sires that drastically affects final pregnancy rate (33.6 to 54.5%). Similar results have been reported in dairy cows, where pregnancy loss (between d 38 and 90 of gestation) was reported from 3.2 to 17.6% across sires used for AI (López-Gatius et al., 2002). Markusfeld-Nir (1997) did an epidemiological study combining more than 50,000 dairy cow pregnancies sired by 233 different bulls and reported that pregnancy loss (from d 45 of gestation until calving) was significantly greater in cows inseminated with semen from 8 specific bulls. Although the average of pregnancy loss of all cows was 5.9%, the incidence of pregnancy loss for these 8 bulls ranged from 10.6 and 17.9%.

In the present study, there was considerable variance in incidence of pregnancy loss among cows mated with different sires that allowed sire

classification as either high or low pregnancy loss. Cows receiving semen from high pregnancy loss sires had 1.9 times greater rate of pregnancy loss, similar to results found by López-Gatius et al. (2002) in dairy herds in which cows receiving semen from high pregnancy loss sires had 3.4 times greater rate of pregnancy loss compared with low pregnancy loss sires during the late embryonic/early fetal period. Pregnancies sired by high pregnancy loss sires also had lower circulating PAG concentrations at d 30 of gestation, even after removing from the analysis pregnancies that were not maintained and are known to have decreased circulating concentrations of PAGs. These findings provide evidence that paternal genetics have considerable influence on pregnancy maintenance in cattle and contribute to the difference in early gestation secretion of PAGs. Investigation of the presence or absence of PAG genes in specific sires may provide clarity to the causes and variance of pregnancy loss, and help develop a selection tool to identify bulls with low embryonic loss.

There are several reports in the literature comparing development of purebred and crossbred embryos. The incorporation of *Bos indicus* genetics has been advantageous to increase the ability of an embryo to tolerate heat stress, specifically in dairy herds, whereas other studies utilize *Bos taurus* genetics in *Bos indicus* herds to increase heterosis and improve embryo development. There is some discrepancy whether these effects stem from the contribution of the oocyte, the spermatozoa, or both. Most of the studies are focused on early development of *in vitro* produced embryos and limited data are reported comparing the effects

of different breed matings in pregnancy maintenance after d 30 of gestation, specifically in beef herds. In the present study, we analyzed pregnancy rate and pregnancy loss using FTAI with Angus and Nelore sires in commercial Nelore cows. Overall pregnancy rate and incidence of late embryonic/early fetal loss (54% and 6.21%, respectively) was comparable with other studies utilizing multiparous Nelore cows exposed estradiol- and progesterone-based FTAI protocols in Brazilian cow-calf operations (Filho et al., 2009; Aono et al., 2013; Pohler et al., 2016a; Pohler et al., 2016b). Nelore cows inseminated with Nelore semen had greater pregnancy rate at d 30 compared with cows inseminated with Angus semen. These results are supported by the idea that *Bos indicus x Bos indicus* embryos are better able to survive elevated temperatures at early stages of development compared with *Bos indicus x Bos taurus* embryos (Barros et al., 2002), given that these cows are very likely to be exposed to heat stress during the breeding season in this region and time of year in Brazil. In contrast, other studies have shown no effect of the genetic background of spermatozoa in regard to the embryo's ability to respond to heat stress (Block et al., 2002). Another explanation for these results is that Nelore sires may have exhibited superior fertility that may have potentially biased pregnancy rate results. This second explanation is not likely because of the equivalent fertility testing at the bull stud facilities. In addition, cows mated with Angus semen had lower pregnancy loss and greater circulating PAGs concentration, indicating a beneficial effect of heterosis in embryo development and pregnancy maintenance in these animals, as seen in

previous studies (Pegorer et al., 2007). Still, these results differ from another study showing the maternal breed effect on PAGs secretion, where cows with *Bos indicus* genetics had increased circulating PAG concentration compared with *Bos taurus* cows (Mercadante et al., 2013). These results led us to believe that PAGs secretion might be related to the embryo genetic composition itself, rather than maternal genotype, but because the present study was not primarily designed to address this observation, we cannot elucidate a conclusion about it.

Estrus expression near the time of FTAI is correlated with pregnancy success, with positive impacts on both ovarian function and uterine environment which affects embryo development and pregnancy maintenance (Sá Filho et al., 2010; Davoodi et al., 2016; Pereira et al., 2016; Pohler et al., 2016b). In this study, estrus expression was evaluated for effects on PAGs concentration, and moreover, to test if pregnancy rate differ among sires when cows expressed estrus before FTAI. Pre-ovulatory estradiol directly affects pregnancy establishment and maintenance through several physiological events including gamete transport and preparation of uterine environment (Hawk and Cooper, 1975; Buhi, 2002; Pohler et al., 2012; Pereira et al., 2016). Our data demonstrated that cows which expressed estrus had 2.7 times greater chance of being pregnant at d 30 than those that did not expressed estrus, similar to previous studies reporting increased fertility when *Bos taurus* cows (Perry et al., 2005; Perry et al., 2007; Thomas et al., 2017) and *Bos Indicus* influenced heifers (Thomas et al., 2017) exhibit estrus before FTAI. Large variation existed among sires with regard to the effect of estrus

on pregnancy rate. For example, cows inseminated with semen from sire AN3 had a 50% increase in pregnancy rate at d 30 when they expressed estrus before or at the time of FTAI, whereas cows inseminated with semen from sire NEL8 the percentage increase was only 4%. In general, cows inseminated with Nelore semen had greater pregnancy rate, independent of estrus expression while pregnancy rate in cows sired by Angus was significantly lower in the absence of estrus before AI. It has been reported that changes in the uterine environment including changes in pH following estrus expression affect sperm transport and longevity (Perry and Perry, 2008), increasing sperm viability until the time of ovulation. Even though there is not a clear reason for this variation among sires, we believe differences may exist in sperm characteristics (genetic or molecular) that explain why some sires seem to be more resilient to changes in the uterine environment compared with others, which may be related with changes in sperm longevity. Although no significant association was detected between estrus score and incidence of pregnancy loss and PAG concentration, previous studies have shown that estrus intensity before FTAI is positively correlated with PAGs concentration during gestation (Pohler et al., 2016b) and future studies would be interesting to address if changes in uterine environment, due to estrus expression, can affect pregnancy maintenance during this period.

Conclusion

In summary, the variation in pregnancy rate and pregnancy loss between sires used for FTAI is significant, yet poorly understood. Cows inseminated with

Nelore sires had greater initial pregnancy rate, but had greater pregnancy loss and lesser circulating concentration of PAGs. Pregnancies by high pregnancy loss Angus sires had lower circulating concentration of PAGs at d 30 of gestation. Exploring the genomic characteristics of these specific sires is the next step in understanding these differences with regard to sire fertility and PAG secretion. Results from this present study may lead toward developing methods to select sires with high pregnancy rate, but low pregnancy loss thus increasing overall reproductive efficiency in the herd.

References

Aono, F., R. F. Cooke, A. Alfieri, and J. L. M. Vasconcelos. 2013. Effects of vaccination against reproductive diseases on reproductive performance of beef cows submitted to fixed-timed AI in Brazilian cow-calf operations. Theriogenology 79: 242-248.

Barros, C. M., F. M. Monteiro, D. S. Mello, L. M. Carvalho, A. B. Teixeira, L. A. Trinca, and E. C. Freitas. 2002. Resistance of Bos indicus to heat shock, compared to crossbred or Bos Taurus, at early stages of in vitro embryo development. In: Proceedings of the International Symposium on Reproduction in Domestic Ruminants, Crieff, Scotland. p A4.

Barton, S. C., C. A. Adams, M. Norris, and M. Surani. 1985. Development of gynogenetic and parthenogenetic inner cell mass and trophectoderm tissues in reconstituted blastocysts in the mouse. Development 90: 267-285.

Block, J., C. C. Chase, and P. J. Hansen. 2002. Inheritance of resistance of bovine preimplantation embryos to heat shock: relative importance of the maternal versus paternal contribution. Mol. Reprod. Dev. 63: 32-37.

Breukelman, S. P., Z. Perenyi, M. A. Taverne, H. Jonker, G. C. van der Weijden, P. L. Vos, L. de Ruigh, S. J. Dieleman, J. F. Beckers, and O. Szenci. 2012. Characterisation of pregnancy losses after embryo transfer by measuring plasma progesterone and bovine pregnancy-associated glycoprotein-1 concentrations. Vet. J. 194: 71-76.

Buhi, W. C. 2002. Characterization and biological roles of oviduct-specific, oestrogen-dependent glycoprotein. Reproduction 123: 355-362.

Davoodi, S., R. F. Cooke, A. C. C. Fernandes, B. I. Cappellozza, J. L. M. Vasconcelos, and R. L. A. Cerri. 2016. Expression of estrus modifies the gene expression profile in reproductive tissues on Day 19 of gestation in beef cows. Theriogenology 85: 645-655.

FASS. 1999. Guide for the care and use of agricultural animals in agricultural research and teaching. Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research, and Teaching.

Filho, O. G. S., M. Meneghetti, R. F. G. Peres, G. C. Lamb, and J. L. M. Vasconcelos. 2009. Fixed-time artificial insemination with estradiol and progesterone for Bos indicus cows II: Strategies and factors affecting fertility. Theriogenology 72: 210-218.

Frade, M. C., C. Frade, M. B. Cordeiro, M. F. Sá Filho, F. S. Mesquita, G. P. Nogueira, M. Binelli, and C. M. B. Membrive. 2014. Manifestation of estrous behavior and subsequent progesterone concentration at timed-embryo transfer in cattle are positively associated with pregnancy success of recipients. Anim. Reprod. Sci. 151: 85-90.

Gatea, A. O., M. F. Smith, K. G. Pohler, T. Egen, M. H. Pereira, J. L. M. Vasconselos, J. C. Lawrence and J. A. Green. 2017. The ability to predict pregnancy loss in cattle with ELISAs that detect pregnancy associated glycoproteins is antibody dependent. Theriogenology.

Green, J. A., T. E. Parks, M. P. Avalle, B. P. Telugu, A. L. McLain, A. J. Peterson, W. McMillan, N. Mathialagan, R. R. Hook, S. Xie, and R. M. Roberts. 2005. The establishment of an ELISA for the detection of pregnancy-associated glycoproteins (PAGs) in the serum of pregnant cows and heifers. Theriogenology 63: 1481-1503.

Hawk, H. W., and B. S. Cooper. 1975. Improvement of sperm transport by the administration of estradiol to estrous ewes. J. Anim. Sci. 41: 1400-1406.

Hill, J. R., R. C. Burghardt, K. Jones, C. R. Long, C. R. Looney, T. Shin, T. E. Spencer, J. A. Thompson, Q. A. Winger, and M. E. Westhusin. 2000. Evidence for placental abnormality as the major cause of mortality in first-trimester somatic cell cloned bovine fetuses. Biol. Reprod. 63: 1787-1794.

Karen, A., N. M. Sousa, J. F. Beckers, A. C. Bajcsy, J. Tibold, I. Madl, and O. Szenci. 2015. Comparison of a commercial bovine pregnancy-associated glycoprotein ELISA test and a pregnancy-associated glycoprotein radiomimmunoassay test for early pregnancy diagnosis in dairy cattle. Anim. Reprod. Sci. 159: 31-37.

Kaufman, M. H., S. C. Barton, and M. A. H. Surani. 1977. Normal postimplantation development of mouse parthenogenetic embryos to the forelimb bud stage. Nature 265: 53-55.

López-Gatius, F., P. Santolaria, J. Yaniz, J. Rutllant, and M. López-Béjar. 2002. Factors affecting pregnancy loss from gestation day 38 to 90 in lactating dairy cows from a single herd. Theriogenology 57: 1251-1261.

Markusfeld-Nir, O. 1997. Epidemiology of bovine abortions in Israeli dairy herds. Prev. Vet. Med. 31: 245-255.

McGrath, J., and D. Solter. 1984. Completion of mouse embryogenesis requires both the maternal and paternal genomes. Cell 37: 179-183.

Meneghetti, M., O. G. S. Filho, R. F. G. Peres, G. C. Lamb, and J. L. M. Vasconcelos. 2009. Fixed-time artificial insemination with estradiol and progesterone for Bos indicus cows I: Basis for development of protocols. Theriogenology 72: 179-189.

Mercadante, P. M., K. M. Waters, V. R. G. Mercadante, G. C., Lamb, M. A. Elzo, S. E. Johnson, D. O. Rae, J. V. Yellich, and A. D. Ealy. 2013. Subspecies Differences in Early Fetal Development and Plasma Pregnancy Associated Glycoprotein Concentrations in Cattle. J. Anim. Sci. 91:3693-3701

Pegorer, M. F., J. L. M. Vasconcelos, L. A. Trinca, P. J. Hansen, and C. M. Barros. 2007. Influence of sire and sire breed (Gyr versus Holstein) on establishment of pregnancy and embryonic loss in lactating Holstein cows during summer heat stress. Theriogenology 67: 692-697.

Pereira, M. H. C., M. C. Wiltbank, and J. L. M Vasconcelos. 2016. Expression of estrus improves fertility and decreases pregnancy losses in lactating dairy cows that receive artificial insemination or embryo transfer. J. Dairy. Sci. 99: 2237-2247.

Perry, G. A., and B. Perry. 2008. Effect of preovulatory concentrations of estradiol and initiation of standing estrus on uterine pH in beef cows. Domest. Anim. Endocrinol. 34: 333-338.

Perry, G. A., M. F. Smith, M. C. Lucy, J. A. Green, T. E. Parks, M. D. MacNeil, A. J. Roberts, and T. W. Geary. 2005. Relationship between follicle size at insemination and pregnancy success. Proc. Natl. Acad. Sci. USA. 102: 5268-5273.

Perry, G. A., M. F. Smith, A. J. Roberts, M. D. MacNeil, and T. W. Geary. 2007. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers1. J. Anim. Sci. 85: 684-689.

Pohler, K. G., T. W. Geary, J. A. Atkins, G. A. Perry, E. M. Jinks, and M. F. Smith. 2012. Follicular determinants of pregnancy establishment and maintenance. Cell Tissue Res. 349: 649-664.

Pohler, K. G., T. W. Geary, C. C. Johnson, J. A. Atkins, E. M. Jinks, D. C. Busch, J. A. Green, M. D. MacNeil, and M. F. Smith. 2013. Circulating bovine pregnancy associated glycoproteins are associated with late embryonic/fetal survival but not ovulatory follicle size in suckled beef cows. J. Anim. Sci. 91: 4158-4167.

Pohler, K. G., J. A. Green, L. A. Moley, K. M. Doran, H. B. Graff, R. F. G. Peres, J. L. M. Vasconcelos, and M. F. Smith. 2014. The effect of embryonic size and sire on circulating concentrations of bovine pregnancy associated

glycoproteins in beef cattle. In: J. J. et al. (eds.) International Symposium on Reproduction in Domestic Ruminants. p 563, Obihiro, Hokkaido, Japan.

Pohler, K. G., M. H. C. Pereira, F. R. Lopes, J. C. Lawrence, D. H. Keisler, M. F. Smith, J. L. M Vasconcelos, and J. A. Green. 2016a. Circulating concentrations of bovine pregnancy-associated glycoproteins and late embryonic mortality in lactating dairy herds. J. Dairy. Sci. 99: 1584-1594.

Pohler, K. G., R. F. G. Peres, J. A. Green, H. B. Graff, T. Martins, J. L. M. Vasconcelos, and M. F. Smith. 2016b. Use of bovine pregnancy-associated glycoproteins to predict late embryonic mortality in postpartum Nelore beef cows. Theriogenology 85: 1652-1659.

Reese, S. T., T. W. Geary, G. A. Franco, M. E. Wehrman, P. J. Hansen, T. E. Spencer, and K. G. Pohler. 2016. Pregnancy Associated Glycoproteins (PAGs) in high vs subfertility heifers. Society for the Study of Reproduction Annual Meeting, San Diego, California.

Reese, S. T., M. H. C. Pereira, J. L. Edwards, J. L. M. Vasconcelos, and K. G. Pohler. 2017. Pregnancy diagnosis in cattle using pregnancy associated glycoprotein concentration in circulation at day 24 of gestation. Theriogenology.

Sá Filho, M. F., A. Crespilho, J. Santos, G. A. Perry, and P. S. Baruselli. 2010. Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous

synchronization with progesterone or progestin-based protocols in suckled Bos indicus cows. Anim. Reprod. Sci. 120: 23-30.

Starbuck, M. J., R. A. Dailey, and E. K. Inskeep. 2004. Factors affecting retention of early pregnancy in dairy cattle. Anim. Reprod. Sci. 84: 27-39.

Stice, S. L., N. S. Strelchenko, C. L. Keefer, and L. Matthews. 1996. Pluripotent bovine embryonic cell lines direct embryonic development following nuclear transfer. Biol. Reprod. 54: 100-110.

Surani, M. A., S. C. Barton, and M. L. Norris. 1984. Development of reconstituted mouse eggs suggests imprinting of the genome during gametogenesis. Nature 308: 548-550.

Surani, M. A., S. C. Barton, and M. L. Norris. 1987. Influence of parental chromosomes on spatial specificity in androgenetic----parthenogenetic chimaeras in the mouse. Nature 326: 395-397.

Thomas, J. M., J. W. C. Locke, B. E. Bishop, J. M. Abel, M. R. Ellersieck, J. V. Yelich, S. E. Poock, M. F. Smith, and D. J. Patterson. 2017. Evaluation of the 14-d CIDR-PG and 9-d CIDR-PG protocols for synchronization of estrus in Bos indicus-influenced and Bos taurus beef heifers. Theriogenology 92: 190-196.

Thompson, I. M., R. L. Cerri, I. H. Kim, J. A. Green, J. E. Santos, and W. W. Thatcher. 2010. Effects of resynchronization programs on pregnancy per artificial insemination, progesterone, and pregnancy-associated glycoproteins in plasma of lactating dairy cows. J. Dairy Sci. 93: 4006-4018.

Wallace, R. M., K. G. Pohler, M. F. Smith, and J. A. Green. 2015. Placental PAGs: gene origins, expression patterns, and use as markers of pregnancy. Reproduction 149: R115-126.

Zoli, A. P., L. A. Guilbault, P. Delahaut, W. B. Ortiz, and J. F. Beckers. 1992. Radioimmunoassay of a bovine pregnancy-associated glycoprotein in serum: its application for pregnancy diagnosis. Biol. Reprod. 46: 83-92.

CONCLUSION

A role that is often downplayed beyond initial fertilization, the sire influences all stages of pregnancy, including embryo mortality. Without collecting extensive field data, fertility cannot be predicted accurately using current in vitro semen analysis. Developing methods to predict a bulls' potential fertility for pregnancy establishment and maintenance without first breeding hundreds of cows is essential to improve reproductive efficiency. Bulls exhibit significant variation in conception rate, pregnancy loss and calving rate. In comparing to breeds, Nelore sires have increased pregnancy rates at day 30 but undergo greater pregnancy loss later in gestation compared to Angus sires. Sire fertility was influenced by estrus expression in the cow. Circulating PAG concentration is decreased in pregnancies sired by bulls with high rates of pregnancy loss. Genomic PAG profiles may provide greater insight to differences in fertility between bulls. Using PAGs as a marker to evaluate fertility may be a novel tool to identify and select high fertility sires that current semen evaluations fail to detect.

VITA

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