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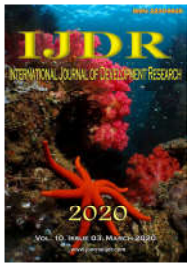

REVIEW ARTICLE

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CONSIDERATIONS FOR THE INTERPRETATION OF BIOCHEMICAL PROFILES IN NON-INFECTIOUS ABORTIONS COWS

[1]Paula Gädicke, [2]Ricardo Chihuailaf, *[1]Reinaldo Letelier and [1]Álvaro Ruiz

[1]Universidad de Concepción, Facultad de Ciencias Veterinarias, Chillán, Chile
[2]Universidad Católica de Temuco, Dpto. de Ciencias Veterinarias y Salud Pública, Temuco, Chile

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****Corresponding author:* *Reinaldo Letelier,***

ABSTRACT

Objective: Abortion in a dairy herd is a factor that impact the company's productivity. It is important to provide epidemiological surveillance tools that help anticipate herd reproductive losses in order to correct those through management measures, infectious disease control and proper nutritional management. The analysis of biochemical profiles as a preventive medicine tool is useful to detect metabolic imbalances that can be part of the causality of non-infectious abortions. In this review we analyzed some indicators of the biochemical profile that may reflect metabolic imbalances of energy and/or protein and minerals that may reflect a risk of non-infectious abortion. The use of this tool has allowed to identify the negative energy balance and liver integrity affected by fat mobilization as risk factors for abortion in cows. Also, cholesterol surveillance, as a protection factor, is recommended, and together with body condition score evaluation, would be a useful tool for evaluating energy imbalances in dairy herds.

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INTRODUCTION

There are several management tools available to accurately control, evaluate and diagnose a dairy farm's health, such as the use of productive and reproductive records, as well as laboratory tests (biochemical profiles). When these factors are analyzed and used in the correct way, it allows us to detect a system's main flaws. Identifying weaknesses and critical points enable us to take corrective measures, thus increasing the company's productivity. Abortion is a significant factor in the loss of cows, with an impact of 30 to 40% losses of total replacements, and an even greater impact when applied to animals with remaining useful life or animals of great genetic value within the herd (Gädicke *et al*., 2010). Thus, the control and monitoring of infectious and non-infectious diseases which can trigger abortions in the herd is of vital importance; not doing so results in heavy consequences, such as the decreases in milk production, potential replacements and decreases fertility, as well as increase in feeding costs, medical treatments, artificial inseminations and slaughter rates (Benavides *et al*., 2010; Abdalla *et al*., 2017). In dairy herds, abortions syndrome creates limitations for production given the economic losses it entails (Markusfeld-Nir, 1997; Gädicke& Monti, 2013).

That can occur sporadically, endemically or in the form of an outbreak, and can be of infectious or non-infectious origin, which makes it difficult to establish the cause in many cases(Carpenter *et al*., 2006). The infectious origin (diseases such as bovine viral diarrhea, leptospirosis, brucellosis, neospora, among others), and non-infectious origin (placentitis, vasculitis, thermal stress, among others)(Gädicke *et al*., 2010), can act both independently and collectively, reflected in the so-called Bovine Abortion Syndrome (BAS), (Gädicke& Monti, 2008). Thus, in order to design tools that anticipate risk, we looked for early indicators related to biochemical alterations. It has been noted that metabolic alterations associated to nutrition and energy required by the animals negatively affects herd reproductive performance, as is the case with high milk productions systems and cystic ovaries Erb *et al*., 1985). Along the same line, Gröhn *et al*. (1990) indicate that increases in individual milk production, with respect to previous lactation, rises the risk of abortion, placental retention, metritis and silent estrus. Additionally, a decrease in body condition can increase losses from 40 to 90 days of gestation, due to a decrease in progesterone production by the *corpus luteum* (Moore *et al*., 2005). In this review we analyzed some indicators of the biochemical profile that may reflect metabolic imbalances of energy and/or protein that may reflect non-infectious abortion risk.

DISCUSSION

Given that abortion has a multifactorial causality, it is defined as a syndrome, which means that variables must be analyzed together and during the periods od times when the highest incidence of abortions is recorded. The fact that all metabolites were inter-related shows a relationship between infectious and non-infectious factors causing BAS. Etiological identification in cow abortion is a difficult task for veterinarians. An integrated approach is essential for the successful diagnosis of fertility and/or abortion problems in a herd (Gädicke & Monti, 2008). The multivariate relationship with BAS etiology is reinforced by 2008-2011 data from the Sub-department for Epidemiological Surveillance in Chile's Agricultural and Livestock Service (SAG – Servicio Agrícola y Ganadero)(SAG, 2011). Among the possible causes of non-infectious abortion, many factors can influence its presentation, including: trauma, toxic plants, toxins, genetic factors, extreme temperatures, stress, maternal endocrine imbalance, placental dysfunction, gestational twinning, inadequate handling, mineral deficiencies (selenium, calcium, magnesium, iron, copper, potassium, sodium and zinc) and nutritional deficiencies(Rivera, 2001; Luna & Roldan, 2013; Norman *et al*., 2012). In the studies carried out on herds from the south-central region of Chile, we have found groups with protein-energetic metabolism imbalances related to nutrition and inflammation, and another's with nutritional protein imbalances, organ damage and high antibody titers for *Neospora caninum* (Keshavarzi *et al*., 2017; Ceciliani *et al*., 2012).

Acute phase proteins correlate with abortion, which is because the immune system's first response to noxious stimuli is a local inflammation. Therefore, when infections and tissue injuries exceed local defenses, the organism reacts by activating a broad systemic response (Eckersall, 2010; SAG, 2011). Various researchers have analyzed and studied the acute phase proteins associated with infectious and inflammatory diseases in certain livestock populations, with the main objective being to characterize the application of these responses. This application has been used – generally along with other markers – to monitor stimulation of the innate immune system. These are tools for monitoring health and diagnosing diseases in production animals (Eckersall, 2010). Therefore, to generate protein response, there must be some condition associated with eventual abortion, such as vasculitis, poor *corpus luteum* function, thrombosis, antigen antibody reaction among other thing (Newcomer *et al*., 2017; Borel *et al*., 2014). A variety of hormonal and metabolic signals from the liver, pancreas, muscle and adipose tissue act on brain centers in order to regulate food consumption, energy balance and metabolism (Erb *et al*., 1985). Since this regulation can influence in the gestation, research has found that metabolic alterations associated with nutrition and energy requirements negatively affect reproductive performance in herds (Moore *et al*., 2005).

When analyzing biochemical profiles throughout gestation, it was observed that the main metabolic imbalances at the herd level in the dairies analyzed were related to energy metabolism, liver integrity and protein metabolism. Aborted cows presented greater alterations in the form of negative energy balances and liver integrity affected by fat mobilization. Aborted cows also presented a more marked lack of synchrony between energy and degradable proteins in the rumen (Gädicke, 2016).

Cholesterol plasma concentrations were lower in of aborted cows than non-aborted cows. This was associated with a protective effect (OR = 0.61 $P < 0.05$) for the probability of abortion, reflecting that cows with higher cholesterol levels (always within the RI) would be less likely to abort (Gädicke, 2016). This metabolite can be considered as a nutritional indicator in cattle (Coppo *et al*., 2016). From a physiological point of view, this may reflect that certain cows adapt differently to productive demand changes and that some are more susceptible and cannot handle metabolic imbalances. Cholesterol plasma concentrations depend on diets, and especially on the type and amount of fat consumed. They increase when intakes are rich in fats, especially unsaturated fats, and decrease in states of malnutrition (Coppo *et al*., 2016). According to Duffield *et al*. (2009), the ability of cows to manage energy intake and demand in critical production periods is the most important aspect in maintaining healthy and productive lactation. A poor response to energy requirements can lead to a multitude of problems, including clinical illnesses and low milk production. It also indices cows at a higher risk of developing metabolic disorders (Cameron *et al*., 1998; Drackley, 1999; Hert, 2000; Shin *et al*., 2015). An indicator of this poor adaptive response is elevations in ketone body concentrations (Hert, 2000), or ketonemia.

Within these metabolites is β-hydroxybutyrate, whose plasma concentrations at second month in aborted and non-aborted cows were within the RI, indicating a process of adipose tissue mobilization due to a significant energy imbalance at the herd level (Fernandes *et al*., 2012). According to some authors, certain blood metabolites such as non-esterified fatty acids (NEFA) are useful in measuring energy balances in ruminants, since they indicate fat mobilization and are more sensitive indicators of negative energy balances(Jackson *et al*., 2011; Wathes *et al*., 2007; Ruprechter *et al*., 2018). Negative energy balances can also be reflected in negative variations of body condition. A higher percentage ($P<0.05$) of our aborted cows had lower body conditions (more than 0.5 points on the BCS scale), compared to the other group of non-aborted cows, month before in which the abortions occurred. This coincides with authors indicating that the loss of postpartum body conditions seems to affect the course of later pregnancy(López-Gatius *et al*., 2002), and that the animals with lower body conditions have higher gestation losses compared to cows with higher body conditions(Thangavelu *et al*., 2015). Logistic regression also indicates that body condition variations are protective factors for abortion in months 3 and 4 of gestation (OR = 0.32, $P<0.05$).

Bloodurea interpretations in ruminants depend on protein and energy consumption, demonstrating that organisms do not have a strong mechanism of homeostasis for maintaining constant levels of urea in the blood, making it difficult to obtain normal reference levels (Álvarez, 2001). We found cases of cows that aborted presented levels notoriously above the RI (Gädicke, 2016). This was possibly due to increased protein consumption, or to diets with easily digestible protein or high levels of non-protein nitrogen, where there was greater absorption of the ruminal ammonia, and therefore greater urea synthesis in the liver. In the study conducted by Piccione *et al*.

(2012), urea concentrations showed a statistically significant increase in the final gestation stage, compared to the other stages and at the beginning of lactation and compared to the remainder of lactation. It has been reported in a study on smaller ruminants that during the lactation period, urea concentrations increase due to higher energy requirements; at the end of gestation, which concentrations clearly depend on protein intake(Wittwer, 2012). In addition, high urea plasma concentrations affect the uterine environment. This can cause a toxic metabolic effect, leading to embryonic death (Wittwer, 2012; Eckersall; 2000; Gädicke; 2009), which can be a significant risk factor in early gestation stages. However, negative energy balances do not affect energy, protein, mineral and hepatic metabolisms of some animals, due to cow should tolerate physiological and nutritional changes, since they are regulated by homeostasis (Wittwer, 2000).

Total protein levels in analyzed farms were maintained within the RI during the sampling months, and 25% of aborted cows had low total protein concentrations between gestation months 3 and 7(Gädicke; 2016). Studies indicate that total proteins increase as breastfeeding progresses, and the opposite happens during the dry period, in which there is a slight decrease. According to Piccione *et al*.(2012) there are maternal physiological requirements for supporting fetuses and providing immunoglobulin's in milk during the lactation period. Hypoproteinemia can occur in malnutrition cases (Wittwer, 2012; Eckersall; 2000).

Albumin is a negative acute phase protein. Its concentrations decrease in response to injury, unlike globulins that increase their levels during the final phase of gestation, near delivery (Wittwer, 2012). According to Kaneko *et al*. (1997), these types of proteins are sensitive to nutritional influences when nutrients are low and dehydration processes were elevated. However, these changes are often subtle and difficult to detect and interpret. Plasma globulins increase in inflammatory or infectious processes. In our studies, plasma concentrations of albumin and globulin in some abortion cases were associated with inflammatory processes. The herds in general had high values of fibrinogen, which is one of the acute phase proteins that is positive for inflammation, possibly indicating non-specific inflammatory processes (Gädicke; 2009; Gädicke; 2016).

Regarding hepatocellular integrity, reflected in the enzymatic activity of aspartate aminotransferase (AST), values in aborted cows exceeded the RI, accounting for damage to the liver and muscles. This triggers a release of bradykinin, histamine, prostaglandins E and F2α, causing luteolysis (Kaneko *et al*., 1997; Ferrando &Urquieta, 1982). In addition, this hepatocellular damage in aborted cows may be secondary to fat mobilization (Kaneko *et al*., 1997) which was a problem in the analyzed herds from southern Chile (Gädicke; 2016). Macromineral metabolites analyses reflect that calcium and phosphorus plasma concentrations in aborted cows were within the RI, although the herd experienced concentrations under the RI. It has been recorded that cows with serum calcium >2.0 mM had lower postpartum NEFA serum concentrations than cows with concentrations <2.0 mM (González, 2000; Reinhardt *et al*., 2011) indicating that normocalcemic cows have better energy balances than subclinical hypocalcemic cows. Cows are prone to developing alterations in serum concentrations of minerals such as calcium and phosphorus in the beginning of lactation, causing diseases such as hypocalcemia and hypophosphatemia. Nutritional strategies are based on manipulations of endocrine control points by means of the absorption mechanism preparation and resorption of macromineral metabolism, so that cows can more efficiently manage the period of negative mineral balances associated with the beginning of the lactation (Wittwer, 2000; Reinhardt *et al*., 2011). Selenium metabolism, estimated by Glutathione peroxidase (Gpx) blood activity, constitutes one of the essential micronutrients, with an adequate contribution being necessary for health maintenance and the reproduction of ruminants (López-Alonso *et al*., 1997),since many authors show that animals require important levels of selenium during the reproductive stage due to metabolic processes of developing organisms that produce large amounts of free radicals as intermediates (Zachara*et al*., 1989; Weiss, 1998). Fat-soluble vitamin plasma concentrations, since retinol and tocopherol are relevant in the immune response against viruses protection. In our studies retinol and tocopherol curves during pregnancy for aborted cows did not differ from non-aborted cows (p> 0.05). However, a median and negative correlation was found (r = -0.42, p <0.05) between retinol plasma concentrations and ELISA results for BVD, which may indicate a protective effect of this vitamin for said disease in the herds analyzed. According to Weiss(1998) the concentration of these vitamins in dairy cows depends on feed sources, management systems and feed availability, since rumens tend to metabolize many of these fat-soluble vitamins.

Conclusion

The analysis of biochemical profiles as a preventive medicine tool is useful to detect metabolic imbalances that can be part of the causality of non-infectious abortions. The use of this tool has allowed to identify the negative energy balance and liver integrity affected by fat mobilization as risk factors for abortion in cows. Also cholesterol surveillance, as a protection factor, is recommended, and together with body condition score evaluation, would be a useful tool for evaluating energy imbalances in dairy herds. It is important to facilitate farmers the use of epidemiological surveillance tools that aid the anticipation of reproductive herd losses through management measures, infectious disease control and adequate nutritional management.

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