

Avanços da pesquisa e inovação e do empreendedorismo em medicina veterinária 2

Alécio Matos Pereira
Ana Larissa Pereira da Silva
Davy Frazão Lima
(Organizadores)



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APRESENTAÇÃO

A domesticação de animais levou o ser humano a conviver diretamente com inúmeras espécies, sendo que algumas delas se tornaram dependentes dessa correlação. A domesticação nos passou a responsabilidade de manter a saúde, nutrição, conforto e segurança dos animais de convívio domiciliar e os destinados a produção, o estudo da ciência animal fornece o conhecimento necessário para manter as melhores condições de vida para esses animais.

O Médico Veterinário e Zootecnista são profissionais que se dedica ao estudo desses animais, com a finalidade não somente de evitar, mas também identificar a nutrição adequada, estudar e tratar patologias que podem afetar diretamente no tempo e qualidade de vida das espécies domesticadas.

Este livro irá complementar os conhecimentos do leitor em diversos aspectos da sanidade animal, auxiliando o corpo acadêmico e profissionais da área veterinária na resolução de quadros clínicos, e indicando alternativas de tratamento.

Em 17 capítulos o livro discorre assuntos na saúde e reprodução de equinos, bovinos, caprinos, cães e gatos, visando apresentar os temas sob os aspectos técnicos e científicos, levando sempre em consideração a didática na apresentação dos conteúdos. Boa leitura!

Alécio Matos Pereira
Ana Larissa Pereira da Silva
Davy Frazão Lima


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OXIDATIVE STRESS: A HIDDEN ENEMY FOR THE OVINE REPRODUCTION

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Víctor Hugo Parraguez

Facultad de Ciencias Veterinarias y Pecuarias
Facultad de Ciencias Agronómicas, Universidad
de Chile

Francisco Sales

INIA-Kampenaiké, Chile; Departamento de
Producción y Sanidad Animal

Óscar Alejandro Peralta

Facultad de Ciencias Veterinarias y Pecuarias

Antonio González-Bulnes

Facultad de Veterinaria, Universidad Cardenal
Herrera-CEU, CEU Universities, Valencia,
España

ABSTRACT: Sheep reproduction is, in general, very efficient compared to other livestock species. However; raising sheep in territories with low nutrient supply or with conditions leading to maternal hypoxemia puts reproduction at risk by increasing birth and perinatal mortality rates. This becomes even more critical in multiple pregnancies, including twin pregnancies, due to the low birth weight of the lambs. In this work, new information is reported regarding the role of fetal oxidative stress resulting from maternal undernutrition and twin pregnancy and its detrimental effects on intrauterine growth in sheep. The supplementation of antioxidants as a strategy to reduce the negative effects of oxidative stress, which usually occurs in twin pregnancies and/or maternal undernutrition, is also discussed. Any

attempt to enhance reproductive and productive performance in twin pregnancies, makes more feasible the implementation of managements leading to increase prolificacy as a strategy to improve the efficiency of sheep farming.

KEYWORDS: twin pregnancy, intrauterine growth restriction, oxidative stress, antioxidants.

INTRODUCTION

Sheep is a highly relevant specie in Latin America and in the world in general, due to its adaptability, hardiness and ability to produce meat, wool, milk and leather, under different environmental and management conditions. These characteristics have resulted in sheep production to be largely relegated to more marginal territories from the agricultural point of view, where other animal production systems, such as cattle farming, cannot be efficiently developed.

Although it is true that, in general, the ovine specie is very efficient from the reproductive point of view, when the herds are kept in marginal areas, the reproductive indices can be diminished or cannot be improved to reach their potential, either due to changes in gametogenesis and steroidogenesis, reduced fertilizing capacity, as well as the incapacity to maintain adequate conditions for implantation and/or embryo-fetal development.

The increase in prolificacy is a strongly developed strategy in countries where sheep

production is carried out in favorable environmental conditions, and where the sheep industry is gravitating towards the national economy. However, this strategy has become a complex issue. On one hand, increasing prolificacy is highly profitable, since the cost of an additional lamb from a twin pregnant female does not exceed 60% of the total cost of having an additional female to gestate that additional lamb in the herd. However, one of the negative and possibly risky aspects for this strategy is that multiple pregnancies result in intrauterine growth restriction, hypoxemia and oxidative stress, so that the average birth weight of twins is substantially lower than that of lambs from singleton pregnancies, with the consequent higher risk of perinatal mortality (Nash *et al.*, 1996) and potentially lower postnatal growth.

Chilean Patagonia, possess a very harsh environment, with low temperatures and limited rainfall during the year, limiting pasture growth and nutrient availability. Despite, more than 50% of the national sheep are raised in this area. Managements leading to increase prolificacy, through the increase in twin pregnancies, is, in general, a practice resisted by producers, since it is associated with high perinatal mortality and decreased profitability. Nevertheless, under these natural management conditions, between 10 and 40% of the pregnancies correspond to twin gestations. For this reason, in recent years we have been studying the causes of intrauterine growth restriction in twin pregnancies and some strategies to counteract the resulting constraints, in order to improve birth weight, neonatal survival and postnatal growth rate.

This chapter addresses some of the factors that limit fetal growth, with a special focus on twin pregnancies developed under restricted environmental conditions, which significantly reduce intrauterine growth. Emphasis is also given to hypoxia-induced oxidative stress, as an important factor which participates surreptitiously in reducing fetal growth, and is normally not taken into consideration.

Factors involved in intrauterine growth restriction (IUGR) in sheep

In single or multiple sheep pregnancies, IUGR may result from a variety of situations associated with decreased maternal supply of nutrients and oxygen to the fetus. In forage-based systems, sheep breeding is often carried out under conditions of undernourishment or frank malnutrition. This situation is aggravated in southern or northern territories, where pregnancy takes place at a time of the year, when there is less supply of forage in natural grasslands, as a consequence of the seasonality of sheep reproductive activity.

As previously mentioned, sheep farming in Chile is concentrated in the Magallanes region (Patagonia), where large seasonal photoperiod changes and low environment temperatures result in limited pasture growth, leading to the fact that most of the gestational period occurs under a naturally-occurring nutritional restriction. There is a broad consensus that, in sheep, the most critical time points during gestation in which maternal undernutrition may lead to a reduced fetal growth, are the peri-implantational and the end of pregnancy

periods. In the peri-implantational period, due to impaired attachment of fetal cotyledons to maternal caruncles, the placentome formation is diminished, leading to lesser placental development and thus placental insufficiency. A reduced functional capacity of the placenta is typically associated with poor placental vascular development, which in addition to prevent adequate nutrition to the fetus, also impairs oxygen delivery, resulting in a hypoxic and nutrient deprived fetal environment. The last third of gestation is critical, as ~90% of fetal growth occurs during this stage, with the consequent increased demand for nutrients, and where any maternal nutrient deficiency may be reflected in the fetus (Igwebuike, 2010).

In several countries of Latin America and around the world, many of the sheep flocks are located in high altitudes. These conditions result in maternal exposure to hypobaric environments, which significantly decrease the fraction of oxygen in the air inspired by the mother. Maternal hypoxemia translates into reduced oxygen supply to the fetus, due to the decrease in the maternal- fetal oxygen gradient that governs gas diffusion in the placenta. Furthermore, exposure to environments rich in atmospheric pollutants or some concomitant pathologies can also lead to hypoxemia, as observed in cardiovascular diseases (anemia, hypertension or heart failure) and/or lung diseases that decrease gas exchange (pneumonia, edema, bronchitis, emphysema, etc.). Whatever the origin of chronic maternal hypoxia, one of the main consequences is the fetus failing to reach its genetically determined growth potential (Hutter *et al.*, 2010). Notably, regardless of the reduction in nutrient supply, hypoxia alone has been shown to have a significant impact on fetal growth, emphasizing its role as a key contributor to intrauterine growth impairment (Parraguez *et al.*, 2005; Soria *et al.*, 2013).

In addition to the direct effect of hypoxia on fetal growth, an indirect effect mediated by oxidative stress has also been established. In previous investigations developed by our group, working with single-bearing ewes under maternal hypobaric hypoxia (at 3600 meters above sea level), we have found that the resulting newborn lambs, characterized by low weight and height, have concomitantly high levels of oxidative stress, and these negative effects on fetal growth are largely prevented by maternal administration of antioxidant vitamins (Parraguez *et al.*, 2011). Interestingly, under the same environmental conditions, maternal hypoxemia and oxidative stress affected placental and fetal development from before the middle of the gestational period. Therefore, the use of antioxidants early in gestation, resulting in an increase in fetal antioxidant status, also plays a preventive role (Parraguez *et al.*, 2015), opening a new window for maternal targeting interventions.

Role of oxidative stress in IUGR due to twin gestation and/or maternal undernutrition

In multiple pregnancies, IUGR is a common condition due to competition between fetuses for limited nutrients, oxygen, and uterine space (Gootwine *et al.*, 2007). In addition, both the individual number of placentomas and the total placental mass is proportionally reduced (van der Linden *et al.*, 2014). All the aforementioned contribute to twin-born lambs

to have a significantly higher risk of neonatal mortality (Nash *et al.*, 1996).

Some evidence suggests that, in sheep, twin pregnancies are accompanied by fetal hypoxia, in the absence of other concomitant conditions (Rurak y Bessette, 2013). Given the scarcity of information in the literature regarding twin pregnancies with IUGR and the eventual role of hypoxia and oxidative stress in this condition, we have recently carried out some studies in this regard. We also considered the effect of undernutrition, due to the frequency of this condition in extensive sheep systems and the evidence that maternal malnutrition has a greater impact on fetal growth in twins compared to singletons (Rumball *et al.*, 2008). In fact, a study carried out at sea level, where the ewes were kept in a natural pasture, covering 72% of their energy requirements but only a 30% of crude protein requirements, showed that at 140 days of gestational age (7 days before delivery), ewes were normoxic, but the condition of twins itself, resulted as an inducing factor of fetal hypoxemia and oxidative stress. However, a maternal nutritional restriction also leads to fetal hypoxemia, but without achieving a significant increase in fetal biomarkers of oxidative stress, regardless of the litter size. In addition, both the twin status and nutritional restriction led to decreased body weight and fetal size. Fetal liver weight showed the same effects, however, brain weight was not affected by either twinning or maternal nutritional restriction, augmenting the brain/liver ratio, which is indicative of IUGR. Although total placental weight was increased, the placental weight per fetus was significantly decreased in twins. Placental weight in nutritionally restricted animals was slightly decreased, but did not achieve statistical significance. The ewes in this study decreased their body condition during gestation, and slightly reduced their body weight from day 70 of pregnancy, which means that they mobilized their reserves to maintain gestation until term (Sales *et al.*, 2018).

In previous studies in sheep kept at 3600 m altitude, we had described that oral supplementation with vitamins C+E, from 30 days before mating until the end of gestation, significantly increased maternal plasma concentrations of these vitamins and prevented the detrimental effects of oxidative stress induced by maternal hypoxemia on the weight of newborn lambs (Parraguez *et al.*, 2011). To provide an insight regarding the role of oxidative stress on intrauterine growth, considering twin gestation and undernutrition as triggering factors, we conducted a study under the same conditions as previously reported at sea level, but in this case, the pregnant ewes were daily supplemented with antioxidant vitamins (C+E), from the time of pregnancy confirmation (via ultrasound at day 30 post mating), until the near term of pregnancy.

By analyzing the different maternal-fetal variables, also at 140 days of gestation, we confirmed that oral supplementation with vitamins lead to an increase in their concentrations in maternal plasma. In addition, we found that the concentration of vitamins C and E in fetal umbilical blood was significantly increased, both in singleton and twin gestations, but with a much lower concentration than those found in maternal blood. Concomitantly, total antioxidant capacity was increased in fetuses of mothers treated with the vitamins. Interestingly, the

concentration of both vitamins in twin plasma was significantly higher than those of the singletons, which could be due to the effects of oxidative stress on angiogenesis; umbilical blood flow, in the case of vitamin C; and the overexpression of specific transporters in the case of vitamin E. Another interesting result of this study is that there were high and significant correlations ($r > 0.7$; $p < 0,001$) between maternal and fetal umbilical plasma concentrations for both vitamins. Likewise, we found that supplementation with vitamins C+E increases fetal weight, both in singleton and in twin fetuses, in natural occurring undernourished pregnancies, but the magnitude of the effect is superior in twins. Finally, it was confirmed that twins have a lower placental weight for each fetus, compared to singletons, however, vitamins supplementation resulted in no effect on this trait. Nevertheless, when estimating placental efficiency (gr of fetus/gr of placenta), it was observed that it is increased by both effects, being a twin as well as vitamins supplementation (Sales *et al*, 2019).

In a latter study in twin-bearing ewes kept under the same natural nutritional restriction, the effects of food supplementation (to cover the requirements of twin gestation) and/or antioxidant supplementation, were tested. Each treatment (feed or antioxidants supplementation) individually increased the birth weight of the lambs by 14.6% and 12.5%, respectively, without statistical difference between groups. In other words, the increase in the antioxidant capacity of the maternal- fetal unit during the gestation of twin-bearing ewes with nutritional restriction, through supplementation with antioxidants, allowed the lambs to present a birth weight similar to that obtained by lambs from well-fed mothers during gestation (Parraguez *et al.*, 2020).

CONCLUSION

In sheep, oxidative stress is a condition associated with twin pregnancy. This condition partially explain the IUGR and is aggravated when twin pregnancies occur concomitant with maternal undernutrition, which impairs fetal growth. Maternal supplementation with antioxidants allows to reduce or limit the occurrence of fetal oxidative stress and the associated detrimental effects on intrauterine growth. The implementation of feasible maternal targeting interventions with antioxidants either in multiple pregnancies, in territories with a low supply of nutrients and/or that induce maternal hypoxemia, could increase lamb birth weight and, consequently, decrease neonatal mortality and potentially enhance the postnatal growth rate of the offspring. The main impact of these findings is that they provide an alternative to enhance the outcome of twin pregnancies under harsh conditions and, as a result, allowing the increase in the sheep industry's efficiency and profitability.

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