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Dystocia and stillbirth in beef cattle with subclinical hypomagnesemia: a case study

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Abstract
We describe the occurrence of dystocia and stillbirths in Brangus and Aberdeen Angus cows and heifers within the context of mineral deficiencies in a beef farm located in Buenos Aires province, Argentina. Water, forage, and blood samples were collected for chemical, serology, and mineral quantification, respectively. Birth weight, sex and presentation of calves were monitored at birth, and calving ease was evaluated. A stillborn Aberdeen Angus female was examined postmortem. The occurrence of dystocia and subsequent stillbirths was recorded in 6.11% (32 out of 523) of dams with subclinical hypomagnesemia. During calving, dystocic animals exhibited prolonged labor, stillbirths, and delivery assistance. No difference in birth weight was registered between dystocic and eutocic births. A postmortem examination of a stillborn calf revealed low hepatic copper concentrations and congenital *Neospora caninum* infection. However, no histopathological findings consistent with *N. caninum* infection were observed, confirming only the congenital infection. Maternal hematological analyses indicated suboptimal levels of calcium, magnesium, and copper. Mineral supplementation with $MgCl_2$ + MgO reduced the likelihood of dystocic calvings in cows. Additionally, non-infectious causes of perinatal deaths were serologically identified in the dams. Dietary analysis showed no mineral-interfering factors. While dystocia is common, its association with mineral deficiencies is likely underreported. Further research is needed to better understand and mitigate the negative impact of mineral deficiencies on the sustainability of beef production systems in the region.

Key words: languid calving, perinatal deaths, mineral deficiencies, mineral supplementation.

Distocias y natimortos en bovinos para carne con hipomagnesemia subclínica

Resumen. Describimos la ocurrencia de distocias y natimortos en vacas y vaquillas Brangus y Aberdeen Angus en el contexto de deficiencias minerales, en un establecimiento situado en la provincia de Buenos Aires, Argentina. Se recolectaron muestras de agua y forraje, así como sangre de madres, para estudios bioquímicos y serológicos, respectivamente. Se analizó el peso al nacer, sexo y presentación de los terneros al nacer, y se evaluó la facilidad de parto. Un natimorto Aberdeen Angus hembra fue examinado *post mortem*. La aparición de distocia y natimortos posteriores se registró en el 6,11% (32 de 523) de las madres con hipomagnesemia subclínica. Durante el parto, los animales distócicos exhibieron trabajo de partos prolongados, natimortos y asistencia en el parto. Todos los natimortos tuvieron buena presentación. No se registró diferencia en el peso al nacer entre los nacimientos distócicos y eutócicos. La necropsia de un natimorto reveló bajas concentraciones de cobre hepático e infección congénita por *Neospora caninum*. Sin embargo, no se observaron hallazgos histopatológicos compatibles con la infección por *N. caninum*, lo que confirma solo la infección congénita. Los análisis hematológicos maternos indicaron niveles subóptimos de Ca, Mg y Cu. La suplementación mineral con MgCl2 + MgO redujo la probabilidad de partos distócicos en las vacas. Además, no se identificaron serológicamente causas infecciosas de muertes perinatales en las vacas. El análisis dietético no mostró factores que interfirieran con los minerales. Si bien la distocia es común, es probable que su asociación con deficiencias minerales esté pobremente reportada. Se necesitan más investigaciones para comprender mejor y mitigar el impacto negativo de las deficiencias minerales en la sostenibilidad de los sistemas de producción de carne de la región.

Palabras clave: partos lánguidos, muertes perinatales, deficiencias minerales, suplementación mineral.

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INTRODUCTION

Reproductive efficiency in livestock systems is crucial for meeting the growing demand for animal protein driven by human population growth. Meat-producing countries must enhance their efficiency to reduce their carbon and water footprints (Reese et al. 2020). Identifying the causes of low weaning rates in ruminant systems is essential for developing prevention strategies to control abortions and perinatal deaths (Cantón et al. 2022).

Difficulties during calving lead to significant economic losses in the farming industry, with the dystocia complex causing a considerable number of calf deaths and reduced reproductive performance (Nix et al. 1998). In Argentina, livestock farming predominantly occurs in extensive pastoral systems, where dystocia is the primary cause of reproductive losses related to non-infectious factors (Campero et al. 2003). Hypomagnesemia is a major metabolic disease affecting beef cattle worldwide (Hindman 2023) and is particularly prevalent in grazing livestock systems (Cantón et al. 2021, Doncel et al. 2021). Magnesium (Mg) is vital for numerous biological functions and lacks specific regulatory mechanisms, necessitating daily intake to maintain adequate levels (Houillier 2014). Environmental and physiological factors can interfere with Mg absorption and bioavailability (Martens et al. 2018).

Hypomagnesemia is endemic in central Argentina (Cantón et al. 2021) and is a leading cause of death in grazing beef cows in other southern hemisphere countries such as Uruguay, Australia, and New Zealand (Doncel et al. 2021). In the province of Buenos Aires, hypomagnesemia accounts for half of all metabolic diseases diagnosed on beef farms and is the primary cause of death in adult cattle (Cantón et al. 2021). Hypocalcemia is one of the most common metabolic diseases in dairy cattle (Lean et al. 2023) but is rare in beef cattle. In contrast, subclinical hypomagnesemia and hypocalcemia are likely underreported in cattle (Melendez et al. 2022). Both hypocalcemia and hypomagnesemia have been linked to reduced colostrum quantity and quality, placental retention, dystocia, and decreased neonatal vitality in dairy cattle (Kovács et al. 2023, Lean et al. 2023). Furthermore, another study in dairy cattle showed that low serum levels of Mg were associated with dystocia, endometritis, placental retention, and lower reproductive performance (Jeong et al. 2018). For its part, copper deficiency, a condition of global significance, predominantly affects growing ruminants (Sakhaee and Kazeminia 2011).

In studies carried out in Argentina, it was found that the administration of inorganic copper during late gestation was associated with an increase in copper concentrations in calves from birth to 160 days of age and a tendency towards weight gain during the first 75 days (Rodríguez et al. 2021). In severe copper deficiencies, outbreaks of spontaneous fractures have been observed in both beef and dairy cattle (Mogaburu Masson et al. 2021, Wehrle-Martinez et al. 2023).

Although dystocia and hypomagnesemia are highly frequent events in the central zone of Argentina, to the best of our knowledge, no studies have evaluated mineral parameters in beef cattle experiencing dystocia. This work aims to describe the occurrence of dystocia and stillbirths in beef cows and heifers with the context of mineral deficiencies in the herd.

MATERIALS AND METHODS

The outbreak occurred during the winter of 2022 on a beef farm located in Lobos (35°13 35°13'47"S 58°51'47"W), Buenos Aires province, Argentina. The affected herd consisted of Brangus and Aberdeen Angus cows and Aberdeen Angus heifers. Reproductive management included fixed-time artificial insemination (FTAI) in August 2021, followed by natural breeding with low-birth-weight bulls with a bull to cow ratio of 5-6% for 60 days. Before mating, the heifers were weighed and gynecologically examined, with only those showing good genital development selected for mating. They were inseminated at 15 months of age using semen from bulls selected for low birth weight. Thirty days after the bulls were retired, an ultrasound was performed, where a general pregnancy rate of 90% was registered in both herds, resulting in 384 pregnant cows and 139 pregnant heifers. The herd was free of brucellosis. One month before breeding, all females received two doses of a commercial vaccine targeting Bovine alphaherpesvirus 1 (BoAHV-1), Bovine viral diarrhea virus (BVDV), *Campylobacter fetus fetus*, *Campylobacter fetus veneralis*, *Leptospira interrogans* Pomona and *Histophilus somni* (Bioabortogen®H, Argentina). The bulls were free from sexually transmitted diseases, including *C. fetus* and *Tritrichomonas foetus*. The semen quality used for FTAI was thoroughly tested.

During the calving season, the dams were in moderate to good body condition (scoring 2.5 to 4 on a scale of 1 to 5) and were grazing in ryegrass (*Lolium multiflorum*) pastures. The study was carried out during the calving season from May to July 2022. Oral Mg supplementation was administered two months leading up to calving, with 25 g of MgO per animal per day added to native grassland hays and in trays. The addition of $MgCl_2$ to the water source at a concentration of 0.5 g L^{-1} started on July 23rd, 2022.

Sampling procedures were conducted in accordance with the standard operational procedures recommended by the Institutional Committee for the Care and Use of Experimental Animals at the National Institute of Agricultural Technology (INTA CeRBAS), Argentina. Water and forage samples were collected on July 23rd, 2022, and subjected to physical and chemical analysis. Forage samples were taken by handplucking method. Different subsamples of forage were taken from each paddock to subsequently obtain a representative sample (pool) of each paddock. The samples were dried in ovens at 60°C until constant weight and ground. Subsequently, wet mineralization was carried out with a mixture of nitric and perchloric acid (3/4) (v/v), using 3 parts nitric acid and 4 parts perchloric acid in a volume-to-volume ratio. The concentration of Ca, Mg and potassium (K) was estimated using atomic absorption spectroscopy (AAS, Perkin Elmer AAnalyst 700, CT, USA) as described by Cseh and Crenovich (1996). Total solids, sulphate, Ca^{++}, Mg^{++}, Cl^-, Na^+ and F^- were measured on the water samples, being within the acceptable reference parameters for consumption. Blood samples were taken on July 23rd, 2022, before the inclusion of $MgCl_2$ treatment in water from dams that presented stillbirths (n = 10) and from pregnant dams in the same herd (n = 9). Blood samples were obtained in pairs by jugular vein puncture. These samples were used for hemogram, serology, and mineral quantification. EDTA was applied to the samples for blood count and for serology and mineral quantification they were centrifuged for 15 minutes at 15,000 rpm. The blood count was performed using a hematology analyzer (Mindray BC-2300) measuring hematocrit, erythrocytes, leukocytes, hemoglobin, and relative and absolute leukocyte formula (reference values: Kaneko 1989). After verifying the calibration curve with a standard serum, serum Mg, Ca, and Cu levels were measured using AAS. Serum P content was estimated by the technique described by Cseh et al. (1994). The mineral profile of the different samples was determined in the biochemistry laboratory (EEA-INTA, Balcarce, Argentina) with all chemical determinations carried out in duplicate.

During the calving season, birth weight, sex and presentation of calves were monitored at birth, and calving ease was evaluated as described by Kovács et al. (2023). Briefly, the degree of dystocia was classified using a 4-point scale: (1) eutocia; (2) light (prolonged spontaneous calving, more than 2 hours from hooves appearance to delivery, and/or assisted calving by one person without the use of mechanical traction); (3) mild (assistance by two people with moderate force without mechanical traction); and (4) moderate dystocia (assistance by three people with considerable force or the use of mechanical extraction).

An Aberdeen Angus female stillborn was examined postmortem. Samples were taken from the lung and abomasal fluid for bacteriology analysis, and from the spleen, lung, and brain for virology and parasitology, as previously described (Campero et al. 2003, Morrell et al. 2019, Cantón et al. 2022). Additionally, samples from the lung, heart, liver, kidney, spleen, thymus, adrenal gland, tongue, lymph nodes, brain, cerebellum, rumen, reticulum, abomasum, and small and large intestine were fixed in a 10% buffered formalin solution for histological analysis. Finally, a liver sample was taken for biochemistry and hepatic Cu concentration was evaluated by AAS.

All analyses were performed using R statistical software, version R-4.3.1 (R Core Team 2015). Descriptive statistics were used for data visualization, and linear models were applied to investigate relationships between dystocia scores, birth weight, dam category, and calf sex. Variability in birth weight by calf sex was assessed using summary statistics. Pearson's χ^2 test with Yates' continuity correction examined the association between dam category (heifer or cow) and stillbirth occurrence. Odds ratios were calculated to determine the likelihood of abortion based on dam category and to assess the relationships between calf birth weight and type of calving (eutocic or dystocic), and $MgCl_2$ supplementation and type of calving. Additionally, odds ratios were estimated to evaluate the relationship between dam seropositivity for various reproductive diseases and the occurrence of reproductive loss.

RESULTS

Calving difficulties (dystocia) were observed in 6.11% (32 to 523) of dams, marked by prolonged delivery times and increased assisted calving rates: 4.43% in multiparous cows and 10.79% in heifers. Stillbirths occurred in 32 cases of dystocia. Most cases (22 out of 32) involved mild dystocia (degree 3), while the remaining cases (10 out of 32) required light assistance (degree 2) from one person after more than two hours from hoof appearance to delivery. All stillbirths associated with dystocia were delivered in good presentation (anterior longitudinal), position (dorso-sacral), and posture (both legs, head, and neck extended). Calves born to cows with either dystocic or eutocic calvings did not differ in birth weight (34.4 ± 1.209 kg and 36 kg ± 0.285 kg, respectively; p = 0.20). Similarly, calves born to heifers showed no differences in birth weight regardless of the ease of calving (29.6 ± 0.856 kg, and 28.4 ± 0.364 kg, respectively; p = 0.19).In dystocic births, no differences in average birth weight between male and female calves were observed (32.4 ± 1.38 kg and 31.5 ± 1.47 kg, respectively; p = 0.65). But stillbirths born from cows were heavier at birth than those born form heifers (34.38 ± 1.38 kg and 29.6 ± 1.47 kg, respectively; p = 0.02). Nevertheless, when the total number of births is analysed, calves born from multiparous cows weighted 20% more than those born from heifers (35.8 ± 0.263 kg and 28.6 ± 0.497 kg, respectively; $p<0.001$). No additional clinical signs or mortalities were recorded among the dams.

Based on the treatment with $MgCl_2$, two groups were formed, represented by 254 dams receiving MgO (107 heifers and 172 cows) and 269 dams receiving $MgCl_2$ + MgO (32 heifers and 212 cows). Following the addition of $MgCl_2$ to the water source, the remaining dams experienced mainly eutocic calving (Figure 1). The use of $MgCl_2$ + MgO reduced the likelihood of dystocic calving by 6.5 times ($p<0.001$). Multiparous cows supplemented with $MgCl_2$ + MgO had a greater probability of eutocic calving (OR = 4.11, $p<0.001$) compared to cows before $MgCl_2$ supplementation. However, heifers did not show a significant increase in calving ease after supplementation with $MgCl_2$ + MgO (p = 0.73). Weight of calves born from cows did not differ before or after $MgCl_2$ addition (36.2 ± 0.375 kg, and 35.7 ± 0.360 kg, respectively; p = 0.35), and the same was observed for calves born from heifers (28.4 ± 0.527 kg, and 29.1 ± 1.03 kg, respectively; p = 0.4). It was found an association between category of the dam and occurrence of dystocia [x2 = 6.13, p = 0.013; stillbirths in cows 17 (4.43%) and heifers 15 (10.79%)], and the odds of stillbirth is significantly increased when the dam is a heifer (OR = 2.61; 95% CI = 1.174 – 5.733; p = 0.012).

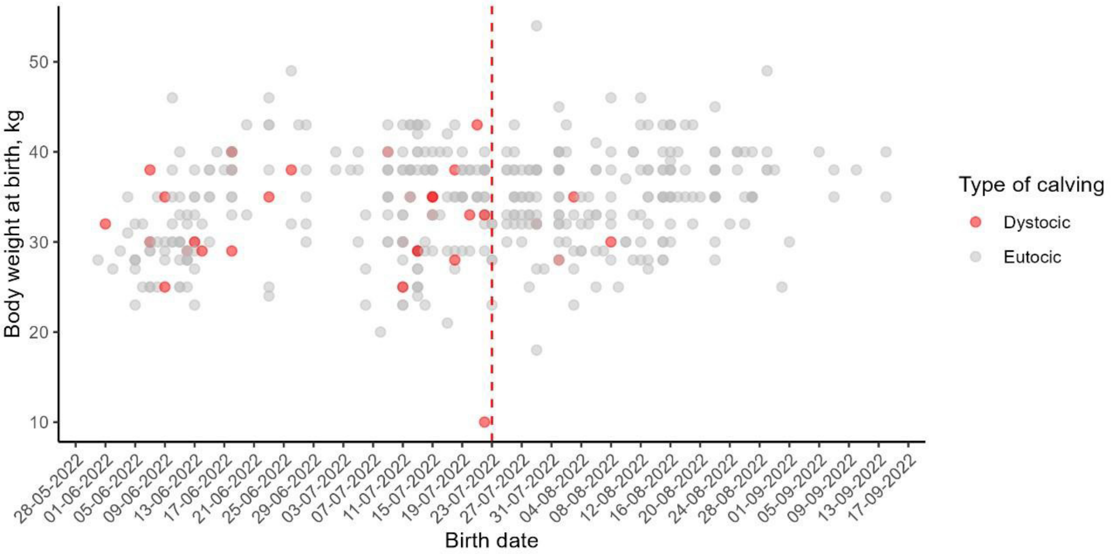

Figure 1. Relationship between the MgO or MgO + $MgCl_2$ supplementation and the occurrence of dystocia. The red vertical dashed line indicates the start date of MgO + $MgCl_2$ supplementation. Body weight at birth is plotted against the birth date, with points colored according to the type of calving: dystocic (red) or eutocic (gray).

All the dams analyzed had good body condition (3.16 ± 0.33). Hemograms for all dams were normal. Some dams with dystocia and pregnant dams exhibited high antibody titers for BVDV and BoAHV-1, low titers for *L.* Pomona, *L.* Wolffi and *L.* Hardjo, and were seropositive for *N. caninum* (Supplementary Table 1). All sampled dams were seronegative for *Brucella abortus*. No epidemiological association was found between the dam´s seropositivity and reproductive losses ($p>0.05$).

The water quality was deemed physically and chemically suitable for cattle consumption, all below the reference values (Table 1). Forage levels of Ca, Mg, and K were also acceptable for cattle consumption, with a normal tetany-potential ratio (K/Ca+Mg) as shown in Table 2. However, serum mineral concentrations revealed suboptimal levels for both groups analyzed: Ca at 7.94 ± 0.71 mg dL^{-1} (reference value = 8.0 - 12.0 mg dL^{-1}), Mg at 1.60 ± 0.25 mg dL^{-1} (reference value = 1.8 - 2.5 mg dL^{-1}), and Cu at 0.56 ± 0.18 ppm (reference value = 0.6 - 1.5 ppm). Conversely, phosphorus levels were normal at 4.91 ± 0.89 mg dL^{-1} (reference values = 3.5 to 7.5 mg dL^{-1}) (Table 3).

Table 1. Physical and chemical water quality available for the affected herds.

Water	pH	Total solids (mg l^{-1})	Sulphate (mg l^{-1})	Ca^{++} (mg l^{-1})	Mg^{++} (mg l^{-1})	Cl^{-} (mg l^{-1})	Na^{+} (mg l^{-1})	F^{-} (ppm)
Heifers	8.05	1148	180	30.8	85	447	236	1.10
Cows	8.40	782	60	10.6	18	63.2	100	0.62

Acceptable reference values (technique): pH: 6.8-9.2 (potentiometry). Total solids: <7000 mg l^{-1} (gravimetry). Sulphate: <1500 mg l^{-1} (turbidimetry). Ca^{++}: < 200 mg l^{-1} (AAS). Mg^{++}: < 500 mg/l (AAS). Cl^{-}: < 4000 mg l^{-1} (mercury thiocyanate colorimetry). Na^{+}: < 5000 mg l^{-1} (atomic emission spectroscopy). F-: < 2 ppm (ion specific potentiometry).

Table 2. Potassium (K), calcium (Ca), and magnesium (Mg) concentration, tetany-potential (K/Ca+Mg) in the fodders grazed by the three affected herds.

Pasture	K (%DM)	Ca (%DM)	Mg (%DM)	Tetany-potential (K/Ca+Mg)
Heifers	2.11	0.38	0.30	1.20
Cows	2.28	0.43	0.30	1.23
Reference values*	< 3 %	>0.40	>0.20	< 2.25

* Reference values (Cantón et al. 2021).

Table 3. Calcium (Ca), phosphorus (P), magnesium (Mg), and copper (Cu) concentration in serum samples from dystocic and pregnant dams of the same affected herd.

Animal	Dystocia degree	Ca (mg dL^{-1})	P (mg dL^{-1})	Mg (mg dL^{-1})	Cu (ppm)
Dystocic cow #1	2	9.16	5.54	1.88	0.62
Dystocic cow #2	2	7.75	5.15	1.98	0.68
Dystocic cow #3	2	7.38	7.24	1.76	0.49
Dystocic heifer #4	3	8.06	5.20	2.19	0.34
Dystocic heifer #5	3	8.37	4.81	1.50	0.97
Dystocic cow #6	3	8.02	5.28	1.32	0.82
Dystocic cow #7	2	6.98	3.58	1.68	0.71
Dystocic cow #8	3	8.49	5.24	1.65	0.57
Dystocic cow #9	3	8.81	5.96	1.70	0.52
Dystocic heifer #10	3	6.59	5.07	1.51	0.53
Average dystocic dam ± SD		7.96 ± 0.81	5.31 ± 0.92	1.72 ± 0.25	0.63 ± 0.18
Pregnant cow #1	-	7.57	3.79	1.21	0.25
Pregnant cow #2	-	7.54	4.60	1.46	0.60
Pregnant cow #3	-	7.81	4.51	1.29	0.52
Pregnant cow #5	-	8.88	3.36	1.32	nd
Pregnant cow #6	-	nd	4.98	nd	nd
Pregnant cow #7	-	nd	4.26	1.69	nd
Pregnant cow #8	-	nd	4.73	1.52	0.49
Pregnant cow #9	-	7.74	5.11	1.62	0.33
Average pregnant cows ± SD	-	7.91 ± 0.56	4.42 ± 0.59	1.44 ± 0.18	0.44 ± 0.14
General average ± SD	-	7.94 ± 0.71	4.91 ± 0.89	1.60 ± 0.25	0.56 ± 0.19
Reference values*	-	8.0-12.0	3.5-7.5	1.8-2.5	0.6-1.5

nd: not determined. *Reference values (Cantón et al. 2021).

The stillbirth calf, recovered from a cow that experienced mild dystocia and required assistance at birth, weighed 9.6 kg and had a skull-rump length of 59 cm, complete hair coverage, and erupting incisors. Autopsy and histopathology revealed no pathological changes. Hepatic Cu levels were 313 µg g^{-1}, below the reference value of over 400 µg g^{-1}. Bacteriological and virological analyses of the stillborn tissues were negative. However, PCR analysis detected *N. caninum*-DNA in the brain of the specimen.

DISCUSSION

The combination of clinical signs in the dams, along with the absence of significant microbiological and pathological findings in both the stillbirth and the dams, suggests that no infectious agent was involved in these events. Additionally, antibody titers for BVDV, BoAHV-1 and some *Leptospira* serovars were detected in both dystocic and pregnant dams of the affected herd. These antibodies are likely of vaccinal origin since no differences in titers were observed between dystocic and pregnant dams. Consequently, no association between reproductive losses (stillbirth) and seropositivity could be established. Furthermore, low levels of Mg and/or Ca were detected in the animals analysed, indicating a deficiency of these macrominerals in the affected herd, which coincided with the dystocic births.

Dystocia, often caused by various factors, is a frequent contributor to perinatal losses, with malpresentation and birth weight being two of the primary factors (Zaborski et al. 2009). In the central region of Argentina, an analysis of 1,693 births in beef cattle revealed that stillbirths due to dystocia occurred in 5.6% of heifers and 2.3% of cows (Alejo et al. 2000). In beef cattle, it has been estimated that the average birth weight of calves born to 2-year-old cows with dystocia is 4 kg greater than that of calves born without dystocia (Nix et al. 1998). However, no difference in birth weight of dystocic and eutocic calves born from cows and heifers were registered in these herds, which allow us to rule out the influence of birth weight with dystocia. Furthermore, based on the date of pregnancy diagnosis, it is difficult to determine whether the eutocic and dystocic dams initially come from FTAI or natural mating. However, no significant differences were observed in the weight of the calves at birth throughout the study, so the influence of the type of service on the occurrence of dystocia can be ruled out. On the other hand, male calves are generally at a higher risk of dystocia compared to female calves (Nix et al. 1998, Zaborski et al. 2009), however, no weight differences based on calf sex were detected in the herds of the present study. Nevertheless, calves born from multiparous cows weighted 20% more than those born from heifers, aligning with the results reported by Duncan et al. (2023). Despite the weight of the stillborn analyzed being very low, difficulty in calving was observed with the cow experienced, unable to give birth without assistance. Additionally, dystocia affected primarily heifers in comparison with multiparous cows, as it has been previously reported (Nix et al. 1998, Hohnholz et al. 2019). Hypomagnesemia is one of the main metabolic diseases affecting adult beef cattle in the region (Cantón et al. 2021), caused by Mg deficiencies in the forage or factors that interfere with Mg absorption (Hindman 2023). Conversely, hypocalcemia is less documented in beef cattle, though Ca metabolism is influenced by Mg deficiency, mainly due to impaired release and action of parathyroid hormone (Martens et al. 2018). Subclinical hypocalcemia and hypomagnesemia have been described in grazing dairy cattle during calving (Melendez et al. 2022).

Despite confirming hypomagnesemia in the herds included in this study, no deaths or neurological syndromes were recorded in any dam. Hypomagnesemic tetany does not necessarily occur even with low blood Mg levels (Martens et al. 2018). Additionally, the good body condition of the dams in affected herds has been proposed as a predisposing factor to the occurrence of hypomagnesemia (Cantón et al. 2021).

Languid calving observed in these herds could be related to reduced uterine motility due to hypocalcemia (Al-Eknah and Noakes 1989), potentially prolonging the birth process and leading to hypoxia with fatal consequences for the calf. An increased risk of hypocalcemia and dystocia has been noted in multiparous dairy cows (those with more than five births) (Lean et al. 2023). Unfortunately, the number of births in the multiparous cows on this farm was not available. Implementing preventive measures to reduce the risk of subclinical hypocalcemia and subsequent calving difficulties has been suggested (Masoumi Pour et al. 2022). Moreover, Mg butyrate supplementation has proven effective in decreasing the need for calving assistance, improving colostrum quality, and increasing calf viability (Kovács et al. 2023). Despite the laboratory confirmation of hypomagnesemia, the cattle had been receiving MgO in feed. MgO is the most widely used source of Mg in formulated diets for livestock, which contains between 51 and 59% Mg and could also serve as a rumen alkalizing agent (Urdaz et al. 2003). Thus, the addition of Mg oxide probably did not contribute to generating a negative cation-anion difference, which could have impeded Ca absorption.

However, after the addition of $MgCl_2$, a significant reduction in dystocia in cows was observed. The reduction in dystocia cases after $MgCl_2$ supplementation is likely related to the improvement in circulating magnesium levels due to increased consumption of this mineral through water. Additionally, the presence of chlorine in the salt has an acidifying effect on the rumen, enhancing Mg absorption. This also acidifies the internal environment of the animal, which increases intestinal calcium absorption, promotes the removal of calcium from bones, and facilitates the dissociation of calcium bound to plasma proteins, thereby increasing the fraction of free ionic calcium (Schonewille et al. 1999, Gelfert and Staufenbiel 2008). However, chloride in $MgCl_2$ could have laxative effects limiting its inclusion in rations (Henry and Benz 1995, Urdaz et al. 2003). On the other hand, $MgCl_2$ is soluble in water, but has a lower Mg content than MgO, acts as a diuretic in excess and has poor palatability (McCaughan 1992), therefore, its administration with MgO in this case was probably more beneficial than if $MgCl_2$ had been applied alone. Maternal nutritional status, primarily macronutrients, can have long-term impacts on fetal development and neonatal performance (Van Saun 2023). Furthermore, parathyroid hormone stimulates calcium transfer across the placenta and maintains a concentration gradient between maternal and fetal blood and has been hypothesized to be an important fetal growth factor (Riond et al. 1995). Despite this, no significant differences were observed in the weights of calves born to cows and heifers between the two treatments established.

Although the forage analyzed did not show tetany potential, several factors might be compromising Mg absorption and bioavailability in the dams (Hindman 2023, Martens et al. 2018). Mg absorption occurs mainly at the pre-stomach level, with the total amount of Mg absorption being influenced by the ionic composition of the rumen fluid (Martens et al. 1988). Among the main factors, high dietary K has the largest and most consistent effect, with larger depressions at 2.25% K or higher (Fontenot et al. 1989, Urdaz et al. 2003). Although no tetanizing potential was demonstrated, K levels were 2.28% for cows and 2.21% for heifers (Table 2), so they would be at levels that could affect Mg absorption. On the other hand, high ruminal ammonium (NH_4^+) contents have been associated with a reduction in Mg absorption (Martens et al. 1988, Hindman 2023). Nevertheless, an adaptive response can normalize Mg absorption at persistent levels of NH_4^+ (Martens et al. 2018).

Serum total concentration of Mg was found to be below the reference values in both dystocic and pregnant cows of the same herd. In addition, half of the dams showed suboptimal serum Ca values. However, measuring the ionized forms (iCa and iMg) might provide a more accurate prediction of the herd's mineral status (Melendez et al. 2022), though these determinations are not routinely performed. Additionally, a soil mineral analysis could have offered a better understanding of potential mineral deficiencies (Kumssa et al. 2019). Despite Mg being one of the most abundant elements in the body, only 2% is readily available, especially in adult cattle (Stöber and Scholz 2005). Moreover, trace mineral requirements during late pregnancy are the sum of maintenance, pregnancy, and colostrum production needs, as well as maternal growth in the case of heifers pregnant for the first time (Van Saun 2023). Therefore, daily dietary intake is crucial to maintaining physiological levels (Houillier 2014), especially in certain reproductive periods where mineral deficiencies can lead to compromised maternal nutritional status (Van Saun 2023).

Hypocupremia was also detected in these dams. However, serum Cu levels often do not accurately reflect the true status of the herd, making hepatic Cu concentration a more reliable marker (Sakhaee and Kazeminia 2011). A low hepatic Cu concentration was found in the stillbirth. Typically, fetal hepatic Cu levels remain within physiological values even when the dam has low Cu levels (Van Saun 2023). However, fetal survival during the birth may require substantial antioxidant capacity to counteract the anoxic conditions experienced, especially during prolonged labor dystocia (Van Saun 2023). This may explain why hepatic copper levels were below the reference values. The relationship between reduced Cu levels in the dams and stillbirth in this clinical context warrants further investigation.

Finally, the presence of *N. caninum* was confirmed in the stillborn, although no histopathological lesions were observed, indicating a congenital infection, which is highly frequent in many herds in Argentina (Cantón et al. 2022).

CONCLUSIONS

Calving difficulties and stillbirths were observed in cows with subclinical levels of Mg and Ca in their blood, with an apparent response to the addition of $MgCl_2$ in cows. The study primarily highlights the potential importance of Mg which is related to Ca levels at the time of calving in beef cows. Further research in a controlled environment is needed to verify these findings and to develop effective mineral supplementation strategies to prevent perinatal reproductive losses.

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Supplementary

Table 1. Serological results for the detection of specific antibodies against Bovine viral diarrhea virus (BVDV), Bovine herpesvirus type 1 (BoHV-1), *Neospora caninum* (*N. caninum*) and different *Leptospira* spp. serovars in affected and non-affected dams.

Animal	Heifer / cow	BVDV[(1)]	BoHV-1[(1)]	N. caninum[(1)]	L. Pomona[(3)]	L Wolffi[(3)]	L. Hardjo[(3)]	Other Leptospira serovars[(3,4)]
Dystocia#1	Cow	1:32	>1:512	-	1/200	-	-	-
Dystocia #2	Cow	1:64	1:64	+	1/400	1/200	-	-
Dystocia #3	Cow	1:32	1:256	+	1/400	-	-	-
Dystocia #4	Heifer	ND	1:256	-	1/400	-	-	-
Dystocia #5	Heifer	1:256	1:32	-	-	-	-	-
Dystocia #6	Cow	1:32	1:32	-	1/800	-	-	-
Dystocia #7	Cow	1:32	1:64	-	1/400	-	-	-
Dystocia #8	Cow	1:64	1:256	-	-	-	-	-
Dystocia #9	Cow	>1:512	1:128	-	1/800	1/200	1/400	-
Dystocia #10	Heifer	1:8	1:64	+	-	-	-	-
Pregnant #1	Cow	1:32	1:64	-	1/800	1/400	1/200	-
Pregnant #2	Cow	1:16	1:16	-	1/800	1/800	-	-
Pregnant #3	Cow	1:64	1:32	-	1/400	-	-	-
Pregnant #5	Cow	1:32	1:64	-	1/200	-	-	-
Pregnant #6	Cow	1:256	1:256	+	1/200	1/200	-	-
Pregnant #7	Cow	1:64	1:128	-	1/800	-	-	-
Pregnant #8	Cow	Negativo	1:128	-	1/400	-	-	-
Pregnant #9	Cow	>1:512	1:32	-	1/400	1/400	-	-

(1) Serum virus neutralization assays. (2) iELISA. (3) Microscopic agglutination test. (4) Other *Leptospira* serovars tested: *L. castellonis*, *L. canicola*, *L. grippotyphosa*, *L. icterohaemorrhagiae*, and *L. tarassovi*.