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Case Reports

Impact of Managing Presumptive Fetal Membrane Hydrops in a Mare on Fetal Livability

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Abstract: A case of presumptive allantois or amniotic hydrops of the fetal membranes was diagnosed in a young Intracytoplasmic Sperm Injection (ICSI) derived embryo recipient. Hydrops is a rare condition and treatment is usually focused on saving the mare. This case report describes the successful management of hydrops of the fetal membranes diagnosed in an Anglo-Argentine mare at 316 days of gestation, which resulted in a live foal. The mare was treated with broad-spectrum antibiotic therapy (30 mg/kg twice a day sulfadiazine-trimethoprim per os), an anti-inflammatory (flunixin meglumine 1.1 mg/kg once a day Intravenously (IV)), plus progestin's (altrenogest 0.44 mg/kg once daily per os) at day 328 of pregnancy, an immature foal was delivered and was immediately treated with antibiotics (ampicillin 10-20 mg/kg three times a day IV and amikacin 25-30 mg/kg once a day IV), antiacid therapy (ranitidine 1.5-2 mg/kg IV twice a day), as well as hyperimmune plasma and fluid therapy. Oxygen therapy was introduced while the foal was asleep. A commercial IgG blood test confirmed the passage of immunoglobulins. The histological architecture of the placenta revealed that both the amnion and allantois cavities were involved. This report describes one of the few hydrops pregnancy cases reported with a positive outcome for both the mare and foal. Early suspicion was found to be crucial for the positive outcome, enabling rapid hospitalization for the monitoring, treatment, and supportive care of both the mare and foal.

Keywords: Allantoic, Amniotic Hydrops, Fetal Membrane, Hydro-Allantois, Hydramnios

Introduction

Hydrops of the fetal membrane is a very rare condition in all species, including the pregnant mare (Allen, 1986; Christensen *et al*., 2006; Govaere *et al*., 2013; Button *et al*., 2017; de Amorim *et al*., 2018). It is very important to recognize this condition to institute the appropriate therapy and possible hospitalization of the affected animal. Normal allantoic and amniotic fluid in the near-term mare has been quantified to be between 8-15 and 3-7 L, respectively (Arthur, 1969; Vincze *et al*., 2019). In large animals, hydro-allantois is more frequently reported than hydramnios (Roberts, 1986).

The causes of this condition are still poorly understood in all species. A dysfunctional placenta either due to increased fluid production, or decreased transplacental drainage, associated with placentitis, has been suggested (Govaere *et al*., 2013; Roberts, 1986; Hong *et al*., 1993; Vandeplassche *et al*., 1976; Robinson, 2002; Shanahan and Slovis, 2011). Disrupted vascular development that causes placental edema, results in an insufficient transplacental exchange rate and accumulation of fluid, as well as vascular changes (Dini *et al*., 2020). Capillary density in chorionic villi seems to be lower in the hydro-allantoic placenta, associated with abnormal expression of angiogenic and hypoxia-associated genes, leading to tissue hypoxia and edema. Hereditary factors (Allen, 1986; Vandeplassche *et al*., 1976; Oppen and Bartmann, 2001), fetus abnormalities (Vandeplassche *et al*., 1976), umbilical cord torsion, and multiple gestations have also been reported as the most common causes (Allen, 1986; Vandeplassche *et al*., 1976; Oppen and Bartmann, 2001). Hydro-allantois is usually a sudden increase in the abdominal diameter, whereas hydramnios seem to develop gradually over weeks to months. In cows,

hydro-allantois has been reported to be often associated with an apparent structural or functional change in the allantois chorion and its vascularization, leading to fluid accumulation. Hydramnios cases are usually associated with genetic or congenital defects of the fetus. The accumulation of fluid can also be associated with the failure of the fetus to swallow the amniotic fluid. The most common clinical sign reported is sudden abdominal distension. Hydrops is frequently misdiagnosed as other conditions such as gastrointestinal problems or twin pregnancies.

Rectal palpation is diagnostic of hydrops because it can reveal an abnormal fluid-filled uterus; moreover, it is usually not possible to feel the fetus, and the ballottement test is negative. Transrectal ultrasound shows an excessive volume of fluid that prevents the foal from being visualized. A transabdominal ultrasound examination could help to differentiate the two types of hydrops based on the fluid depth range (Bucca *et al*., 2005). In addition, fetal fluid samples can be obtained by ultrasound-guided contests, which provides a definitive differentiation between the two hydrops conditions based on different biochemical reference ranges (Williams *et al*., 1993).

The critical aspects of hydrops are the risks of respiratory distress, ambulatory difficulties, a republic or abdominal wall rupture or herniation, republic tendon rupture (Jalim, 2020), and complications (i.e., hypovolemic shock) because of the large amount of fluid loss during foaling. Due to uterine inertia, dystocia may occur, and the fetus is usually dead at birth or dies postpartum. A common sequela is often reported to be the retention of fetal membranes and septic metritis. Treatment focused on saving the mare due to the often negative outcome of the fetus, while the prognosis of the mare may be good if no major complications occur (Lanci *et al*., 2021) and the mare can be rebred in the same season (Lemonnier *et al*., 2022). To date, only a few cases of long-surviving foals with hydrops have been reported (Christensen *et al*., 2006; Mitchell *et al*., 2019).

This case report describes the successful management of presumptive hydrops of the allantois or amniotic membranes in an Anglo-argentine mare at term which resulted in a live and long-term surviving foal.

Case Report

A six-year-old Anglo-Argentine Intracytoplasmic Sperm Injection (ICSI) derived embryo recipient mare was referred to the Veterinary Teaching Hospital of the University of Pisa on day 316 of pregnancy due to a sudden abnormal increase in abdominal distension, and ventral abdominal edema, which had developed in the previous 24 h. The owner reported no previous episodes of abdominal pain, respiratory distress, or gait abnormalities. Owner-informed consent was obtained at admission.

At clinical examination, the mare was bright and alert, with a body condition score of 4/9 (Henneke *et al*., 1983). Mild tachycardia Heart Rate (HR) 50 beats/min; physiological range: 35-45 beats/min; and tachypnea Respiratory Rate (RR) 28 breaths/min; physiological range: 12-15 breaths/min were recorded. Mucous membranes were pink and moist with a capillary refill time of<2 sec. No lameness or increased digital pulse was found. Abnormal abdominal distension with moderate ventral edema and mild discomfort at palpation of the abdominal wall midline were present. Neither the abdominal wall nor republic tendon tears were evident at the ultrasound evaluation.

Blood samples were drawn from the jugular vein into EDTA and sera tubes for a Complete Blood Count (CBC) and a haemato-chemical panel. The CBC parameters were within reference values, while a mild increase in creatine kinase activity was noted (307 U/L, reference range: 18-217 U/L). This was probably due to rhabdomyolysis secondary to muscle stretching or small abdominal tears not evident at the ultrasound assessment.

On transrectal palpation, abnormal distension of the uterus over the pelvic rim with a non-palpable foal and a negative balloting test were highlighted. The transrectal ultrasound examination revealed a distended uterus with marked edema of the placenta. The combined thickness of the utero-placenta unit was higher than normal in literature for this stage of gestation (Bucca *et al*., 2005). Because of the excessive amount of intrauterine fluid, it was not possible to visualize any of the fetal parts.

On transabdominal ultrasound examination, the fetus was surrounded by a large amount of anechoic fluid and showed a very slight increased HR (83 beats/min) concerning the gestational stage (reference interval: 75±7 beats/min) (Reef, 1998).

Based on clinical examination and diagnostic imaging evaluation, a presumptive diagnosis of hydrops of the allantois and amniotic membranes was made.

Materials, Treatments and Monitoring

At hospitalization (316 days of pregnancy), the mare was submitted to large broad-spectrum antibiotic therapy with sulfadiazine-trimethoprim (30 mg/kg twice a day per so for 10 days), flunixin meglumine for pain control and Systemic Inflammatory Response Syndrome (SIRS) treatment (1.1 mg/kg once a day Intramuscularly [IM] for 10 days), and altrenogest (0.044 mg/kg once a day per os until foaling) to maintain cervical tone and uterine immobility. Cryotherapy was administered using ice packs on the hoof wall (Van Eps and Orsini, 2016) to prevent laminitis and an abdominal bandage was applied (Fig. 1) to prevent excessive weight on the abdominal wall and a secondary rupture.

The fetus's heart rate from day 321 of gestation remained within the physiological range until the day before foaling (Table 1).

Mare clinical parameters, monitored twice a day until foaling, remained within the physiological ranges, except for a mild increase in the digital pulse in the last three days before foaling.

After four days of treatment, the Combined Thickness of the Uterus and Placenta (CTUP) decreased to within the reference range (11 mm) and the CK activity (215 U/L; interval ranges: 18-217 U/L) was also within the reference interval.

A foal alert device was positioned at day 327 of gestation, and the mare was under 24/24-h surveillance.

Peripartum Period

At 00:20 AM on day 328 of gestation, the mare began to foal, expelling a very large amount of mild turbid fluid. The mare showed no signs of severe hypovolemic shock no shacking or ataxia). No intravenous fluid therapy was thus performed during foaling; however, a maintenance IV fluid therapy was administered immediately after foaling due to mild tachycardia (HR 52 bpm).

The fetus was active in the cranio-longitudinal dorso-pubic position and delivered easily. Only mild manual traction was applied to help in the last step which resulted in an uncomplicated delivery with short-term assistance.

The placenta was delivered completely immediately after foaling and was visually examined on both surfaces in an F-shape to check the integrity and gross signs of alteration. The inspection revealed an intact, complete, but slightly thick placenta (involving localized areas of both horns and placental body) (Fig. 2A-B). Both fetal membranes were significantly enlarged (Fig. 2A-B). The umbilical cord was normal in length (54 cm) (interval range: 36-83 cm; average: 55 cm) (Whitwell, 1975). The placenta weighed 7 kg, equal to 14% of the foal Body Weight (BW) (50 kg, using commercial scales, lamas' electronica balance mod. WETOIML, Via I Maggio, 6-43022-Montechiarugolo (PR)-Italy), which is too high (reference value: <11% of the foal BW) (Schlafer, 2004).

Table 1: Foal HR (beats/min) recorded from day 321 of gestation until the day before birth

Gestation day	HR (beats/min)
316	83
321	75
322	65
323	66
324	68
325	66
326	65
327	68

Fig. 1: Mare affected by abdominal enlargement wearing a commercial abdominal support bandage

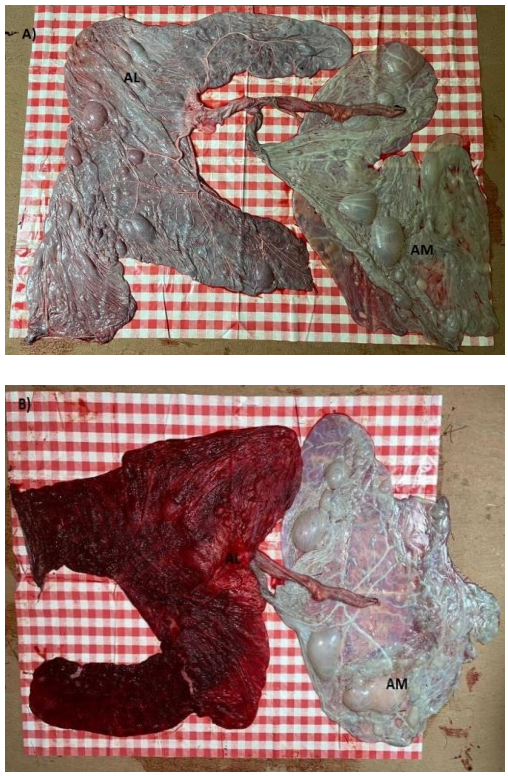

Fig. 2: Fetal membranes. + (A) Allantoic surface (AL). The Amnion (AM) is glossy and enlarged. (B) Allantochorionic surface. Allantocorion is enlarged with a slight increase in thickness

The placenta was sampled in uniform segments collected from each area of the fetal membrane (gravid and non-gravid horn, amnions, and umbilical cord near the insertion of the chorioallantoic sac). All samples were fixed in a 10% formalin buffered solution and routinely embedded in paraffin. Histological sections were stained with hematoxylin and eosin (H and E), and the Van Gieson method.

Colostrum quality was assessed as fair (18%) (Morel, 2020) using the Brix refractometer (Atago Brix N1, Japan) according to the manufacturer's instructions. The mare was treated with levosulpiride (0.5 mg/kg twice a day, IM for 15 days (Chavatte-Palmer *et al*., 2002; Guillaume *et al*., 2003) starting the day after foaling due to poor milk production. The uterus underwent physiological involution, and no uterine clearance problem was recorded. Cycle resumption and ovulation occurred 11 days post-foaling with no fluid accumulation (Katila, 2001).

Results

The Foal

The colt was born 10 days before the expected term and showed an APGAR (appearance, pulse, grimace, activity, and respiration) score of 6/8 at birth (Sgorbini *et al*., 2007). The time to raise the head was within the physiological range (<1 min), while the time to acquire sternal recumbence was delayed and the suckling reflex was weak until approximately 6-7 h after birth. Clinical examination at birth showed body temperature within the reference ranges (38°C; 37.2-38.6°C) (Madigan, 2013), a mild increase in lung sounds, a mild decrease in gut sounds, and an holosystolic murmur audible on the 3rd intercostal space on the left compatible with incomplete closure of the arterial duct. The foal showed soma compatible with immaturity: In particular, flaccid ears, laxity of the flexor tendons, slightly rounded head, thin subcutaneous fat, and slight atrophy of the buttocks.

Immediately after birth, a blood sample was drawn from the jugular vein. An EDTA sample was used to perform a CBC count, while the lithium-heparinized sample was used to perform hem gas analysis using a fully automated blood gas analyzer (ABL 700 series radiometer, radiometer copenhagen medical aps, 2700 Brønshøj, Denmark). The complete blood count revealed increased PCV (44.5%), leukopenia (3.42 K/μL), neutropenia (1.23 K/μL), and lymphopenia 1.81 K/μL). The hemogas analysis showed hyperlactatemia acidosis (lactate 8.5 mmol/L), hypoglycemia (60 mg/dl), and increased creatinine (2.6 mg/dl). A central catheter (14G Long Term Milacath, USA) was placed in the jugular vein and hyperimmune plasma (plasma life Horse, plasma life SRL, Siena, Italy) was administered to prevent failure of passive transfer (fair colostrum quality and weak suckling reflex) and as a colloid to improve dehydration (950 mL administered in 1 h). Fluid therapy (Ringer lactate, 5 mL kg/h IV) was administered immediately after the hyperimmune plasma to correct the metabolic imbalance. Broad-spectrum antimicrobials (ampicillin 10 mg/kg three times a day IV and amikacin 25 mg/kg once a day IV) were applied, together with flunixin melamine (0.5 mg/kg three times a day IV), and an anti-H2 receptor to prevent gastroduodenal ulcer (ranitidine 1 mg/kg three times a day IV). The umbilicus was disinfected using a 2% iodine solution (then repeated once a day for two days). A commercial phosphate osmotic enema was administered to prevent meconium retention. Tetanus antitoxin was administered because the mare booster had not been performed before foaling with the tetanus toxoid vaccine. Dexamethasone was administered (4 mg IM only once) due to suspected dysmaturity (early delivery and soma compatible with immaturity; neutrophil/lymphocyte ratio: 0.68).

The colt was fed using a 14F nasogastric tube (Mila, USA) and from a bowl when the suckling reflex was strong (6-7 h after birth). Approximately 250 mL of colostrum/milk was administered for each meal. The blood glucose concentration normalized after the first meal and remained within the reference range for foals throughout the hospitalization period. The colt was also supplemented with commercial powdered milk (Nanut foal, ACME, Italy) fed from a bowl, and powdered charcoal was also administered (one table spoon/8g, twice daily) to control intestinal meteoric. The milk supplementation was stopped when the mare's milk supply was considered to be sufficient (within the 4th day of birth).

The hypothalamic-pituitary-adrenal axis and neutrophil/lymphocyte ratio were assessed with a low-dose ACTH stimulation test, evaluating the cortisol concentration and CBC before and 1 min after the administration of cosyntropin (10 micrograms). The cortisol concentration increased at least 3-fold after drug administration and the neutrophil/lymphocyte ratio was >2, thus dexamethasone treatment was not repeated.

12 h post-partum, the colt was able to stand with help, but he maintained the quadrupedal position only for a few min due to flexor tendon laxity and he was not able to self-feed. Twenty-four h post-partum, the colt acquired a quadrupedal position more easily with help and was fed from the udder.

At 24 h of age, the IgG concentration was assessed using a commercial ELISA semi-quantitative kit (snap foal IgG, index, USA) and was shown to be >800 ng/dl. Fluid Therapy was discontinued after 5 days of age when creatinine and lactate recovered their physiological values.

A radiographic examination was performed to assess the cuboidal bone ossification and the presence of lung atelectasis. Bone ossification was present, while an alveolar pattern, compatible with pulmonary atelectasis, was found. Oxygen therapy was introduced (6 L/min) during sleep by nasal insufflation until day 2 post-partum when clinical parameters (RR and breathing effort), arterial hemogas analysis, and lung ultrasound evaluation were normal.

24 h post-foaling, the foal began to show intestinal bloating and difficulty in defecating, thus an osmotic enema was repeated three times over 24 h. After the therapy, the meconium was completely excreted, however, meteorism and abdominal pain were not solved, thus an oral laxative therapy was started with sodium sulfate via a nasogastric tube every 24 h. The laxative therapy was stopped after three days when the feces began to be easily expelled. The umbilical remnants were checked by ultrasound at 24 h of life and every three days, as well as lung fields.

The colt was discharged nine days post-partum with normalized CBC and acid-base balance, clinical parameters within physiological ranges, and much-improved flexor laxity. No ultrasound lung abnormalities were present at discharge, nor were heart murmurs, while the umbilical remnant was not completely involved although no signs of infection were present.

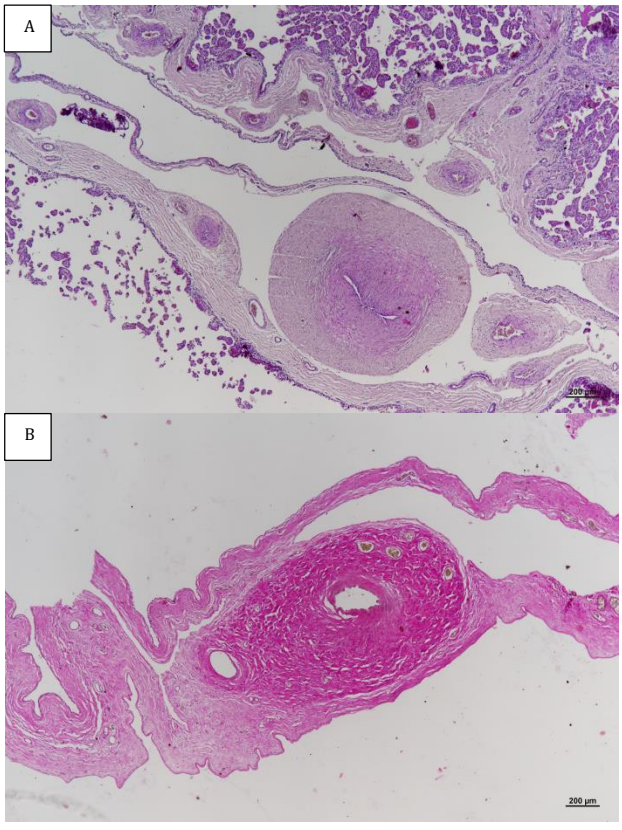

Fig. 3: (A) Gravid horn. Marked thickening of the adventitial layer and narrowing of the lumen of an arteriole. The chorionic villi are morphologically unremarkable (Hematoxylin and eosin, bar = 200 µm); (B) Amnion. Thickening of an amnion arteriole, associated with neovascularization and diffuse edema of the mesenchymal stromal layer (Van Gieson stain, bar = 200 µm)

Placenta Histology and Histological Diagnosis

Both in the non-gravid horn and the gravid horn, chorionic villi, and micro-cotyledons were morphologically normal and quantitatively appropriate. In the gravid horn sample, the arteriolar vascular structures had a larger diameter and narrower Lumina than those of the non-gravid horn, due to the thickening of the adventitial layer (Fig. 3A). This was also observed in the amnion arterioles together with diffuse edema of the mesenchymal stromal layer associated with neovascularization (Fig. 3B). The epithelial layer of the amnion was normal. No inflammatory infiltrates were observed in any of the samples, and the umbilical cord was unremarkable. According to the histological findings, both amnion and allantois cavities were affected. The pathogenesis appeared to be of vascular origin, probably due to decreased transplacental drainage. However, clear evidence of a cause-effect relationship was not demonstrable.

Discussion

We reported the management of presumptive allantois and amniotic hydrops of the fetal membranes in a mare resulting in the birth of a live foal. In the mare, hydrops is a medical emergency that can lead to a republic or abdominal wall rupture or herniation, republic tendon rupture (Jalim, 2020), and hypovolemic shock during foaling (Arthur, 1969). The fetus is usually dead at birth or dies postpartum, and dystocia may occur because of uterine inertia.

In our case report, the presumptive diagnosis of the hydrops of the allantois or amniotic membranes was based on physical and clinical examination, supported by transrectal and transabdominal ultra-sonographic findings.

Currently, differentiating between the types of hydrops is diagnostically limited. Rectal palpation and transrectal ultrasound are considered diagnostic, however, it is still very difficult to identify which cavity is involved. Transabdominal ultrasound can be helpful, but a definitive differentiation is possible via ultrasound-guided amniocentesis and allantocentesis. This could have revealed which of the two cavities was involved by comparing the biochemical reference ranges (Williams *et al*., 1993; Lemonnier *et al*., 2022; Paccamonti *et al*., 1995). Unfortunately, such an analysis was not performed.

According to the literature, hydrops cases usually have a poor prognosis for both the mare and foal due to multiple complications (Christensen *et al*., 2006; Slovis *et al*., 2013; Lanci *et al*., 2021). When no precautions are taken, foals are usually aborted, stillborn or dead during postpartum (Lanci *et al*., 2021;

Abraham and Bauquier, 2021; Foster, 2022; Cantón *et al*., 2023). In addition, abnormalities have been reported (Oppen and Bartmann, 2001), and foals are often premature. In these cases, euthanasia is recommended (Lemonnier *et al*., 2022).

After fetal fluid expulsion, the mare did not suffer from hypovolemic shock. Uterine inertia and placental retention often occur in this condition. In this case, the mare showed no expulsion difficulties of the fetus, and the placental membranes were delivered immediately after foaling. In bovine species, the literature shows contrasting results. According to Roberts (1986), IV infusions were reported to prevent hypovolemic shock during the expulsive foaling stage (Roberts, 1986). However, a more recent study (Elmore, 1992) suggested no need for fluid support because fluid is lost directly from the uterus and not from the systemic circulatory system (Elmore, 1992).

The colt presented immaturity signs but recovered completely after therapy and intensive care support and so far the outcome has been successful (age: 20 Months).

Histological examination of allantois and amnios revealed partially inconsistent findings with those describing hydropic changes in edematous fetal membranes with reduced angiogenesis (Dini *et al*., 2020). The etiopathogenesis is unclear in this case; however, it is assumed to be related to reduced vascular drainage. It is conceivable that the thickening of the vessel walls was the consequence of previous inflammatory changes that were no longer present at the time of the histological examination.

Conclusion

The authors wish to thank students who were likely decisive for the successful outcome of this case. This led to the timely hospitalization of the mare for monitoring, treatment, and supportive care, which ensured a live foal and no injuries or complications to the mare.

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Author's Contributions

Carlotta Bocci, Micaela Sgorbini and Diana Fanelli: Drafted, fieldwork, acquisition of data, drafted the article, read and approved the final version of the manuscript.

Duccio Panzani, Rebecca Moroni and Irene Nocera: Fieldwork, acquisition of data, drafted the article, read and approved the final version of the manuscript.

Çağla Aytaş and Carlo Cantile: Histological examinations and diagnosis, acquisition of data, drafted the article, read and approved the final version of the manuscript.

Ethics

Owner-informed consent was obtained at admission.

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