EQUINE AUTOLOGOUS PLATELET CONCENTRATES: A COMPARATIVE STUDY BETWEEN DIFFERENT SYSTEMS

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Introduction: Autologous platelet concentrates (APCs) are used in horses to promote tissue healing, especially for tendons. Numerous systems, based on several techniques and originally developed for humans, are routinely applied in equine patients. However, preliminary process validation and adequate in vitro biochemical characterization for most systems lack in horses.

Objective: To determine and compare the biological characteristics of equine APCs obtained using four commercial systems and a non-commercial double centrifugation technique.

Methods: Blood samples from six healthy horses were processed to produce APCs using one filtration-based and four centrifugation-based techniques. Platelet, leukocyte, Platelet-Derived Growth Factor-BB (PDGF-BB) and Transforming Growth Factor-β1 (TGF-β1) concentrations were determined in all APCs and compared.

Results: Depending on the technique, cell and growth factor concentrations differed markedly from their baseline values, with significant differences between some systems: median platelet enrichment factor varied between 136% and 516%. Median PDGF-BB enrichment ranged from 166% to 1099%, whereas it varied between 87% and 634% for TGF-β1. Leukocyte concentration strongly decreased in one system, but median enrichment factor varied between 96% and 644% in the others.

Conclusion: Hematologic and biochemical characteristics varied strongly among the APC techniques. These marked discrepancies are supposed to have an impact on clinical outcomes and further studies are needed to determine their influence on the quality of tissue regeneration. Clinicians must be aware of the variability between these systems and should not rely on data relative to human patients when selecting a method for horses.
USE AN EPIDURAL IMPLANTABLE ACCESS PORT SYSTEM TO PROVIDE LONG-TERM ANALGESIA IN EQUINE OSTEOARTHRITIS

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Topic: 7. Equine Medicine and Surgery / Joint or Tendon Disorders

Osteoarthritis is a degenerative disease characterized by chronic pain and one of the major causes of economic losses and welfare impairment on equines. In most of cases an effective analgesic therapy is not feasible due to high costs and long-term side effects. A three years old mixed breed stallion was admitted at Veterinary Medical Hospital with history of progressive lameness in right pelvic limb for 4 months. The diagnosis of proximal interphalangeal osteoarthritis was performed by clinical and radiographic exams. Due to advanced stage of articular degeneration and owner’s financial constraints, it was proposed a palliative analgesic support using an epidural implantable access port system. One 16G epidural catheter was inserted into the epidural space through the sacrococcygeal space and the catheter’s distal end was progressed until the lumbosacral space. The medical appliance (port) was installed beneath the skin, above the superficial gluteal muscle, approximately 10 cm from dorsal midline. The device was easy to implant, with no complications. During 15 days the animal was treated with 0.1mg.kg\(^{-1}\) of morphine through the port and presented substantial decreased of lameness, improved daily comfort and weight bearing. The morphine dosage was gradually decreased. Daily administration can be done in the box which facilitates the box rest. Even the animal staying more time in recumbence than a healthy horse, the chance of spinal infection is nullified by skin protection over the catheter. So, as in humans, epidural technique using an implantable device is an option for long-term chronic pain modulation.
OPTIMIZATION OF TRANSPORT CONDITIONS FOR AUTOLOGOUS BONE MARROW MULTIPOTENT STROMAL CELLS FOR THERAPEUTIC APPLICATION IN HORSES

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Topic: 7. Equine Medicine and Surgery / Joint or Tendon Disorders

Multipotent stromal cells (MSC) have been successfully applied in equine orthopaedics. After isolation and expansion in laboratories, MSC are often transported for several hours until their final use. The aim of the study was to investigate the influence of different transport conditions on MSC quality. Bone marrow was harvested from six healthy horses. MSC were isolated, expanded and exposed to different transport conditions, such as temperature (-80°C; -20°C; 4°C; room temperature), suspension media (phosphate buffered saline; autologous bone marrow supernatant; autologous blood plasma; Cryostor®; Hypothermosol®, fetal bovine serum including 10% dimethyl sulfoxide) and three different concentrations (5, 10 and 20 x10⁶ MSC/ml). Six different commercially available containers were evaluated for MSC biocompatibility (Cryotube; Cellseal™; four combinations of glass-plastic-rubber syringes). To determine the influence of these factors on MSC quality, cell viability (trypan blue staining), dehydrogenase activity (WST-1 test), differentiation capacity (adipogenic, chondrogenic, and osteogenic differentiation) and proliferation capacity were evaluated 24, 48 and 72 hours of exposure to the different experimental conditions.

Our data indicate that different transport conditions affect MSC quality and their biological properties. Temperature, media and time significantly influenced (p<0.05) MSC viability, whereas MSC concentrations did not. Due to containers’ design, significant loss of cells was observed.

Based on our results we recommend that equine bone marrow MSC should be transported using glass syringes with rubber plunger cap, suspended in autologous bone marrow supernatant at 4°C within 24 hours.
A CASE REPORT OF NEUROTOXIC SYNDROME OF LEUKOENCEPHALOMALACIA IN A TWO YEARS OLD COLT

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Leukoencephalomalacia is a disease of horses and donkeys caused by the ingestion of the mycotoxin “Fuminisin” produced by the fungus “fusarium moniliforme”. In June 2011, a two year old colt kept in a feedlot around Karaj was presented for clinical examination. The colt in addition to anorexia, lethargy, somnolence, weakness, recumbency and muscle fasciculations as general clinical signs, presented with a variety of neurological signs such as ataxia, head pressing, circling and leaning to one side. Based on the clinical evidences and the history of ingestion of roughage and grains inappropriately stored, equine leukoencephalomalacia was suspected. At necropsy, no gross pathologic findings were seen in internal organs. Macroscopically, the brain was congested, having hemorrhages and friable consistency. Microscopically, in the white matter of brain, necrosis and degeneration of neurons and vascular damages were apparent. Feed specimens submitted to the laboratory, were diagnosed to be contaminated with Fusarium spp. Finally, the diagnosis of equine leukoencephalomalacia was confirmed according to the above evidences. It is worthwhile to mention that prevention of the occurrence of the disease can be achieved by improving the storage conditions and avoidance of feeding moldy feeds especially moldy corn.
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TREATMENT OF PYTHIOSIS IN EQUINE LIMBS USING INTRAVENOUS REGIONAL PERFUSION OF AMPHOTERICIN B AND DMSO

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Ten horses affected by *Pythium insidiosum* and presenting exuberant granulation tissue in the distal thoracic or pelvic limbs were used in this study aiming to evaluate the effects of the administration of amphotericin B associated with DMSO by intravenous regional perfusion (IRP) after surgical excision and thermocautery. After surgical excision of the granulation tissue and thermocautery, the horses received 50 mg of amphotericin B (10 mL) diluted in a 10% DMSO solution (6 mL DMSO in 44 mL of Ringer Lactate) through a catheter placed in a superficial vein of the affected limb next to the lesion after placing a tourniquet above the injection site. Hematological evaluations were performed and the lesions were evaluated before treatment and seven, fourteen, twenty-one, twenty-eight, thirty-five and sixty days after it. One hundred percent (100%) of horses treated with amphotericin B associated with DMSO showed complete resolution of the lesion in a period between 35 and 60 days after one IRP, depending on the size of the lesion. The IRP technique induced no side effects. All horses presented bacterial infection confirmed by high leukocytosis and neutrophilia before treatment that returned to normal values one week after treatment achieving lower values during the next weeks of treatment. In the same way the anemia diagnosed in all animals of this study was solved during the treatment period. The administration of amphotericin B associated with DMSO by IRP was effective to treat pythiosis in the limbs of horses resolving the infection with no side effects.
DIAGNOSTIC LAPAROSCOPY IN THE RECUMBENT HORSE. CASE STUDY

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A 10-year-old gelding of the Wielkopolska breed was referred for treatment to the Faculty of Veterinary Medicine, the University of Warmia and Mazury. An interview with the owner revealed weakly expressed colic symptoms and decreased appetite.

In view of the symptoms, the horse's relatively satisfactory condition and the clinic's previous experience in dealing with cases of the type, a decision was made to perform diagnostic laparoscopy on suspicion of small intestinal intussusception.

Equine laparoscopy was performed in a recumbent position, the examination of jejunal loops did not reveal any changes suggesting intussusception. The liver contour was enlarged with rounded lobe edges. An enlarged spleen was also observed. Tissue samples were collected from both organs for a histopathological analysis. The time of the laparoscopic procedure, including liver and spleen biopsy, was 45 minutes.

The histopathological test revealed normal spleen structure, and parenchymatous degeneration of hepatocytes was noted in the liver with focal mononuclear cell infiltration. Based on the results of histopathological tests, the patient was referred for internistic treatment. Weak colic symptoms were observed on two more occasions in the course of one week, after which they ceased. Increased appetite and greater mobility were noted following changes in the animal's diet. The causes of parenchymatous degeneration of hepatocytes were not determined. Colic symptoms first appeared towards the end of the three-month pharmacological treatment of an orthopedic injury, suggesting that the administered drugs had an adverse effect on the liver.
Obstruction of the nasolacrimal outflow pathway in horses is not uncommon. Causes of obstruction include foreign bodies, trauma, and congenital abnormalities. A 3-year-old Arabian mare was presented with a history of purulent discharge from the left eye. Ocular examination revealed marked epiphora and mucopurulent conjunctival discharge. The examination was completed following pharmacological mydriasis with 0.5% pro-paracaine hydrochloride. Following application of pro-paracaine, nasolacrimal lavage was completed by cannulation of the upper nasolacrimal puncta with a 24 gauge × 3/4 inch intravenous catheter and flushing with 10 mL of sterile saline. Dacryocystorhinography were completed following administration of xylazine (0.5 mg/kg of body weight). Approximately 4 mL of Renografin-60 (Diatrizoate Meglumine Injection USP) was injected through the upper cannula and lateral and oblique radiographs was completed. Dacryocystorhinography accurately revealed an obstruction of the left distal portion of nasolacrimal duct. The nasolacrimal obstruction was treated by creating a new drainage opening by an incision in the nasal mucosa inside the nostril with placement of a stent for 3 weeks. Post-operative therapy consisted of topical antibiotic/corticosteroid combination, (gentamicin /dexamethasone), q12h for 3 weeks, then q24h until stent removal. In this case, there was no evidence of nasolacrimal obstruction noted after surgery.
DIAGNOSTIC VALUE OF WHOLE BLOOD PROTHROMBIN TIME DETERMINATION IN COLIC HORSES

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Gastrointestinal colic could be concluded in sepsis and/or disseminated intravascular coagulation, thus coagulation parameters (Prothrombin Time /PT/, activated Partial Thromboplastin Time, etc.) could have diagnostic and/or prognostic value. Usability of a whole blood coagulation analyzer was tested, aiming to eliminate long response time of laboratory tests.

Jugular venous blood from 50 colic and 10 non-colic horses were withdrawn once daily and hemostasis tests were conducted prior to surgery, during consecutive post-surgery days and during an observation period in colic, and for 5 consecutive days in non-colic animals. In addition, hemostasis and hematology parameters were measured when taking the anamnesis. Only clinical cases were involved.

A sharp discrimination could be made between surgically and conservatively treated horses based on the PT measurements. Whole blood PT values of surgically treated horses significantly differed from the values of conservatively treated ones (in average 160 % and 116 % of the normal value, respectively).

Plotting whole blood PT values measured during the first 24 hours of the treatment shows difference between surgically and conservatively treated groups. In horses with severe colic signs PT continuously increased exceeding 300 % of the normal value, while PT of conservatively treated horses declined to normal values or to a slightly elevated level (at maximum 130 %). Plasma PT showed no significant differences among the different cohorts.

Our results support the hypothesis that whole blood PT test could be served as a diagnostic/prognostic marker, applicable in colic diagnostics of horses.

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THE EFFECTS OF LIVE YEAST (NCYC SC47) ON FAECAL PH IN HORSES MAINTAINED ON A HAY AND CONCENTRATE DIET

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Laminitis is a common cause of lameness in horses and has far-reaching implications on welfare. Dietary-laminitis appears to be intrinsically linked to rapid fermentation of soluble carbohydrates in the caecum and colon of the horse. Low caecal pH has been cited as the cause of increased intestinal permeability and decreased luminal integrity, as is typical of CHO-induced laminitis. It has been postulated that faecal pH is correlated to caecal pH, and that the measurement of pH within voided material can act as an indicator of acidity in the caecum and colon. The aim of this study was to investigate the effects of live yeast on faecal pH. Faecal pH was measured in a group of eight horses prior to supplementation with live yeast (NCYC Sc47, LFA, France). Horses were maintained on a hay:concentrate diet providing a forage:concentrate ratio of 70:30, and horses were in medium work. Initial faecal pH was measured over 7 days using a pH meter calibrated against both pH 4 and pH 7 buffers. Following the initial measurement period, horses were supplemented with 10g of live yeast/day for 21 days, after which faecal pH was measured again. Data were analysed using a Wilcoxon’s test. Faecal pH was significantly higher (P<0.001) after supplementation with live yeast (NCYC Sc47) and was maintained near to neutral. It is therefore possible to conclude that the live yeast, NCYC Sc47 exerts a buffering effect in the caecum and colon of the horse, which can be measured in the faecal material.
HEMOSTATIC PROFILE IN COLIC HORSES: PRELIMINARY RESULTS

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Background: Colic in horses is a syndrome caused by many reasons – from the less severe which can be treated conservatively (eg. uncomplicated obstipation) to serious ones that require surgical treatment (eg. hernias, volvulus). Due to the alteration of the intestinal wall there is a big risk of endotoxemia. Consequently, tissue damage or endotoxemia can result in alterations of hemostasis.

Objective: The aim of this study is to describe alterations in hemostatic profiles in colic horses.

Methods: Blood was collected from horses referred with colic to Equine clinic UVPS Brno (n=21) at admission into tubes containing citrate and EDTA. Platelet count (PLT), prothrombin time (PT), activated partial tromboplastin time (aPTT), fibrinogen (FBG) were measured and thromboelastography (kaolin-activated) was performed in the samples.

Results: Obtained results were: median PLT 120*10⁹/l (min-max 63-378*10⁹/l), aPTT 56.8 s (13.0-118.7 s), PT 17.5 s (15.2-28.7 s), FBG 1.30 g/l (0.30-6.43 g/l), reaction time 7.4 min (0.7-19. min), clot formation time 2.25 min (1.2-14.5 min), angle alpha 57.7 degrees (19.7-74.9 degrees) and maximal amplitude 63.4 mm (5.2-81.4 mm). Maximal amplitude was significantly lower in horses that underwent surgery (55.3 mm, n=10) than in horses treated conservatively (65.9 mm, p<0.05).

Conclusion: Colics are associated with various changes in hemostatic profiles. Further studies may reveal important factors in initiation of DIC as a complication of this syndrome.

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EVALUATION OF NEGATIVE ACUTE PHASE PROTEINS IN HORSES UNDERGOING NATURALLY AND EXPERIMENTALLY OCCURRING COLIC – PRELIMINARY RESULTS

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As a result of injury, trauma or infection there is a release of mediators that stimulates acute phase proteins synthesis (FAZIO et al, 2010). The levels of total protein and negative APP albumin and transferrin were measured on 12 horses undergoing naturally occurring colic and 15 horses submitted to experimental intestinal obstruction. It was used to date 15 animals allotted in three groups: (G1) natural colic, underwent surgery and survived; (G2) naturally occurring colic treated surgically that died or was sacrificed seven to ten days after surgery; and (G3) healthy horses subjected to colic surgical procedures. Blood samples were collected before (T0) and right after laparotomy (T1), and one (T2), three (T3), five (T4) and seven (T5) days after surgery. Six animals from G1 showed on average values for total protein, albumin and transferrin of T0: 6.15g/dL, 3.72 g/dL and 0.38g/dL; T1: 6.35g/dL, 3.9g/dL and 0.37g/dL; T2: 5.69g/dL, 3.25g/dL and 0.31g/dL; T3: 6.01g/dL, 3.47g/dL and 0.35g/dL; T4: 6.06g/dL, 3.49g/dL and 0.34g/dL; T5: 5.9g/dL, 3.33g/dL and 0.41g/dL respectively. In six animals from G2 it was observed levels of T0: 6.5g/dL, 4.01g/dL and 0.42g/dL; T1: 4.91g/dL, 2.97g/dL and 0.44g/dL; T2: 5.71g/dL, 3.28g/dL and 0.34g/dL; T3: 4.25g/dL, 2.63g/dL and 0.28g/dL; T4: 5.07g/dL, 2.87g/dL and 0.39g/dL; T5: 3.36g/dL, 1.9g/dL and 0.24g/dL respectively. From three of the fifteen G3 animals it was observed, at T0: 7.81g/dL, 4.37g/dL and 0.33g/dL; T1: 7.68g/dL, 4.38g/dL and 0.33g/dL; T2: 8.3g/dL, 4.74g/dL and 0.27g/dL; T3: 7.68g/dL, 4.23g/dL and 0.34g/dL; T4: 7.59g/dL, 4.09g/dL and 0.32g/dL; T5: 8.1g/dL, 4.3g/dL and 0.29g/dL.