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Platelet Lysate as a Novel Serum Free Media Supplement for the Culture of Equine Bone Marrow Derived Mesenchymal Stem Cells

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Mesenchymal Stem Cells (MSCs) produced for clinical purposes rely on culture media that includes fetal bovine serum (FBS). FBS unfortunately is xenogeneic, and thus has the potential to significantly alter the MSCs phenotype, rendering these cells immunogenic. This may result in the rejection of MSCs by the host immune system following administration, even when autologous MSCs are used. Platelet lysate (PL) is considered a possible alternative to FBS that has shown promising results in human and equine medicine. Our goal was to evaluate the use of equine platelet lysate pooled (ePL) from donor horses in place of FBS to culture and expand equine MSCs. We hypothesized that ePL, produced following apheresis, will function as the sole media supplement to accelerate the culture and expansion of equine bone marrow derived MSCs without altering their phenotype and their immunomodulatory capacity. Platelet concentrate was obtained from five equine blood donors via plateletpheresis and ePL was produced via freeze-thaw and centrifugation cycles. Population doublings (PD) and doubling time (DT) of bone marrow derived MSCs (n=3) at P2 to P5 cultured with media supplemented with either 10% FBS or ePL, was calculated using established mathematical equations. Cell viability was assessed via a Live/dead assay and immunophenotypic analysis for the expression levels of MHC-II, CD90, CD105, CD45, CD34 and CD44 markers was performed with flow cytometry. To assess the ability MSCs to modulate inflammatory responses, equine monocytes were stimulated with LPS *E.Coli* and co-incubated with MSCs cultured in the two different media formulations. Following eighteen hours of incubation, cell culture supernatants were collected and assayed for the production of the pro-inflammatory cytokine tumor necrosis factors-alpha (TNF-α). Our results revealed that MSCs cultured in ePL media exhibited increased PDs and decreased DT compared to those in FBS. Moreover, MSCs cultured in ePL showed comparable viability and expressed similar levels of MSCs markers compared to FBS. MSCs cultured in ePL expressed lower levels of CD34 and CD45. Finally, MSCs cultured in ePL efficiently suppressed the release of TNF-α when exposed to LPS stimulated monocytes. Our data demonstrate that ePL supports the proliferation, viability and immunomodulatory capacity of MSCs without altering their phenotypic profile. Thus, ePL has the potential to be used for the expansion of MSCs before clinical application, avoiding the concerns associated with the use of FBS.