

Identifying pZP-immunocontracepted mares using IgG isotypes

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Immunocontraceptive vaccines based on porcine zona pellucida (pZP) antigens have the greatest potential to help the Bureau of Land Management (BLM) maintain horse populations at appropriate management levels. SpayVac® uses pZP in a patented liposome formulation (Immunovaccine, Inc., Halifax, NS, Canada) and has delivered single-dose, long-lasting (4 to 10 years) immunocontraception in several species including deer (*Dama dama*), grey seals (*Halichoerus grypus*), and horses (*Equus caballus*). Current methods to assess contraceptive efficacy in vaccinated mares include observation of pregnancies and/or foaling and determination of pZP antibody levels using ELISA. Previous studies have demonstrated a positive correlation between levels of pZP antibodies produced and contraceptive effect; however, individual mares that were consistently infertile did not necessarily have the highest antibody titers. The objective of this study was to identify potential differences in specific IgG isotype responses among mares treated with aqueous emulsion SpayVac to improve our understanding of vaccine efficacy and potential management applications. 15 of 30 mares vaccinated with aqueous emulsion SpayVac by BLM in 2011 were still infertile 4 years later. We analyzed serum samples collected 1, 2 and 4 years post-vaccination from these mares that were continuously infertile or had foaled at least once during the 4-year period (n=14 each). Additional samples from the continuously infertile mares (n=15) were collected on 24 February 2016 (5 years post-vaccination). A fluorescent bead-based assay was used to distinguish IgG1, IgG4/7, IgG5, and IgG6 isotype responses against pZP. IgG1 antibodies were generally higher in the infertile compared to the fertile mares, but only IgG4/7 antibodies were significantly higher in infertile mares during years 1 and 2 post-vaccination (p<0.05). A paired t-test demonstrated IgG4/7 isotype levels were significantly higher during year 5 compared to year 4 in the continuously infertile mares (p=0.0117; n=15). IgG4 antibodies are often produced in response to long-term exposure to antigen, have anti-inflammatory activity, and they can inhibit the effects of autoantibodies. The liposome formulation likely results in continuous release of pZP antigen, supported by reports of recurring swelling at vaccine injection sites, and preferentially results in production of IgG4/7 antibodies. Measuring IgG4/7 isotypes, in addition to determination of pZP antibody titers, may help identify effectively contracepted mares. (Acknowledgments: Funding from the USGS helped support this research; we thank Butch Roelle and colleagues for samples, and Jennifer Rhode and Heather Freer for assistance in analyses.)