A novel sperm protein deficit identified in two cases of normozoospermic idiopathic infertility in the stallion

A Swegen1 JR Clulow2 RJ Aitken1 Z Gibb1
1Priority Research Centre for Reproductive Science, University of Newcastle, Australia
2Tamworth Equine Veterinary Centre, Scone Equine Group, Australia

Introduction

Absolute infertility in the stallion can be a source of great frustration and significant financial loss across all sectors of the equine breeding industry. Where motile and morphologically normal spermatozoa are present in the ejaculate, and infertility cannot be overcome by artificial insemination, veterinarians are often left with minimal options for further diagnostics and treatment. In this study we report on advanced investigation of complete infertility in a 5-year-old Thoroughbred stallion and suspected complete infertility in a 10-year-old Australian Stock Horse (ASH) stallion, leading to the discovery of a novel deficit in spermatozoa that may open new avenues for diagnosis and treatment of infertility.

Case history

The TB stallion was deemed infertile after performing 72 covers and achieving 0 pregnancies over one breeding season within a closely managed system. The ASH stallion was suspected infertile after serving over 25 mares over multiple breeding seasons (including both unmanaged paddock covers and closely managed AI) without achieving a single pregnancy. Both stallions displayed normal libido, breeding behavior and ejaculation, and semen contained viable, morphologically normal and progressively motile spermatozoa (number of total progressively motile sperm per ejaculate > 1x10⁹). Clinical examination did not reveal any potential causes of poor reproductive performance and the stallions were referred for an advanced sperm function assessment with the aim of identifying a cause for infertility.

Results

Stallions were collected regularly and sperm samples were subjected to a suite of flow cytometric, functional and proteomic analyses, alongside samples from control (fertile) stallions. Procedures were approved by the University of Newcastle’s Animal Care and Ethics Committee (approval number A-2011-122). In both stallions, the majority of spermatozoa had adequate DNA resilience (SCSA) and intact acrosomes (FITC-PNA). Sperm capacitation (assessed by immunoblotting for tyrosine-, serine- and threonine-phosphorylation) was mildly altered but considered adequate. Acrosome reaction competence was assessed following treatment with progesterone or calcium ionophore A23187, and was similar to that of fertile controls. Heterologous zona pellucida binding assay (using bovine oocytes) revealed severely impaired zona binding capacity of spermatozoa collected from the TB stallion; zona binding is currently under investigation in the ASH stallion. To further explain the functional deficit in zona binding, sperm protein expression of the infertile stallions was compared against fertile stallions. Zona Pellucida-Binding Protein (ZPBP) was significantly reduced in spermatozoa of both infertile stallions (p<0.05). Western blotting and indirect immunofluorescence using anti-ZPBP antibody showed that while ZPBP is abundant in spermatozoa from fertile stallions, this protein was consistently under-expressed or absent in the infertile stallions.

Relevance to Australian clinical equine practice

This investigation is the first report of a sperm ZPBP deficit alongside impaired zona binding capacity underlying complete infertility in the stallion. These results demonstrate that advanced sperm assessment options are available to equine veterinarians to assist in investigations of infertility in stallions. Furthermore, diagnosis of zona binding deficits as a
cause of infertility suggests that intracytoplasmic sperm injection (ICSI) can provide a feasible treatment option for valuable stallions in the near future.