Ovarian dynamics & injection site reactions associated with immunocontraceptive zona pellucida (ZP) & GnRH vaccination of domestic horse mares (Equus caballus)

Nolan M.B., Bertschinger H.J., Schulman M.L.
Section of Reproduction, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa

Introduction
Immunocontraceptive vaccines elicit an immune response to endogenous molecules critical to conception and the most extensively evaluated vaccine antigen is the zona pellucida (ZP), particularly porcine ZP (pZP) (Barber & Fayrer-Hosken 2000). The ZP proteins are highly conserved facilitating interspecies use of ZP antigens (Barber & Fayrer-Hosken 2000, Fayrer-Hosken 2008). Determinants of veterinary immunocontraceptive vaccines include ovarian function and injection site reactions.

Materials and methods
The study was conducted in KwaZulu Natal Province, South Africa from November 2016 until May 2017. Mare recruitment depended on confirmation of oestrous activity via trans-rectal palpation and ultrasound examination and a serum progesterone concentration (SPC) > 1 ng/ml. Mares, stratified by body condition scores (BCS Mean (range)), and age (Mean (range)) were assigned to five treatment groups. Groups 1-4 treatments (1 ml total volume) incorporated Pet Gel A (6%) and Poly (I:C) (500 µg) adjuvant in sterile water, in a two or three inoculation protocol five weeks apart, incorporating the following specified antigens: no antigen (Group 1, n=8), BCS 5 (4,6), Age 4 (2,9); 100 µg pZP then 100 µg pZP booster (Group 2, n=7, 6 (5,7), 4 (2,8)); 500 µg recombinant zona pellucida (reZP) then 500 µg reZP and finally 500 µg reZP boosters (Group 3, n=8, 5 (3,6), 4 (2,10)); 100 µg pZP then 500 µg reZP booster (Group 4, n=8, 5 (3,6), 4 (2,7)); 2 ml of 400 µg GnRH-F protein conjugate (Improvac, Zoetis, South Africa) and also as booster (Group 5, n=8, 5 (4,7), 4 (2,7)). Treatments were administered and, or measurements were taken on D0 (December), D35 (January), D70 (February), and D105 (March). Following treatment administration by deep intramuscular injection into the gluteal muscles, ovarian dynamics (via trans-rectal examination and SPC measurements) and injection site changes were monitored at each time point. A composite of three reproductive measurements assessed treatment effects on potential fertility status contingent on satisfying > 1 of the measurements and was examined using a Generalised Estimating Equation Linear Model (IBM, SPSS statistics V.24).

Injection site reactions, assessed by inspection and palpation using a three point scale and rectal temperature were measured for 7 days post-treatment.

Results
The GEE Model detected significant differences between treatment (Tx) groups and time points (P≤0.001). Post-treatment, multiple pairwise comparisons between groups highlighted significant differences in the proportion of mares maintaining potential fertility status between Group 1 and Groups 3 and 5 (P≤0.005, P≤0.001, respectively) and Group 2 and Groups 3 and 5 (P≤0.005, P≤0.001, respectively). Further post hoc pairwise comparisons on the proportion of mares with a potentially fertile status at specific time points were conducted for D70 (Tx 1 + 35 d for Groups 1,2,4 and 5 and Tx 2 + 35 d for Group 3) and D105 (Tx 2 + 35 d for Groups 1,2,4 and 5 and Tx 3 + 35 d for Group 3). Differences were detected between Group 1 and Groups 3 and 5 at D70 (Tx 1 + 35 d) (P≤0.005, P≤0.001, respectively) and Group 2 and Groups 3 and 5 (P≤0.005 and P≤0.001, respectively). Similarly, at D105 differences were detected between Group 1 and Groups 3, 4 and 5 (P≤0.05, P≤0.005 and P≤0.005, respectively) and between Group 2 and Groups 3, 4 and 5 (P≤0.005, P≤0.005 and P≤0.001, respectively). Comparisons between Groups 3, 4 and 5 demonstrated differences between Group 3 at D35 (Tx 1 + 35 d) and Group 4 and Group 5 at D70 (Tx 1 + 35 d) (P≤0.05, P≤0.001, respectively) and Group 3 at D70 (Tx 2 + 35 d) and Group 5 at D105 (Tx 2 + 35 d) (P≤0.05). No differences were evident at D70 between Group 3 and Group 4 (P=0.394) and at D105 between Group 3 and Groups 4 and 5 (P=0.564, P=0.651, respectively).

Conclusions
Suppression of ovarian activity following immunocontraception was:

- similar following initial inoculation and one booster of a reZP formulation, an initial inoculation of a pZP formulation with a reZP booster or with a two treatment pZP formulation protocol;
- superior with a two treatment GnRH vaccine protocol compared to a two treatment reZP protocol.

Literature cited

Acknowledgements
The authors would like to thank the proprietors and staff at Waterford Farm Stud for the provision of animals and assistance with animal handling and data collection.