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

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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 GnRF-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 $\geq$ 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.

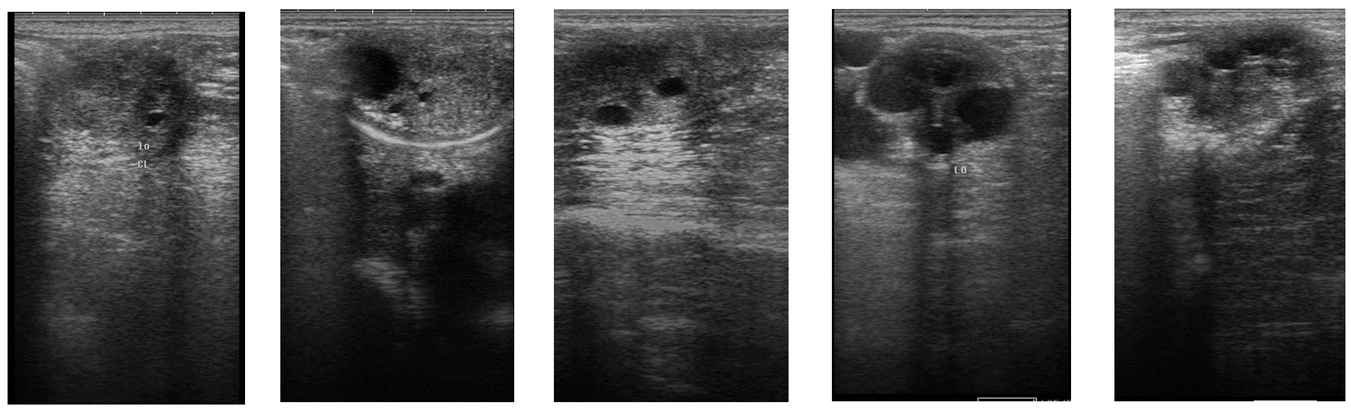
Results

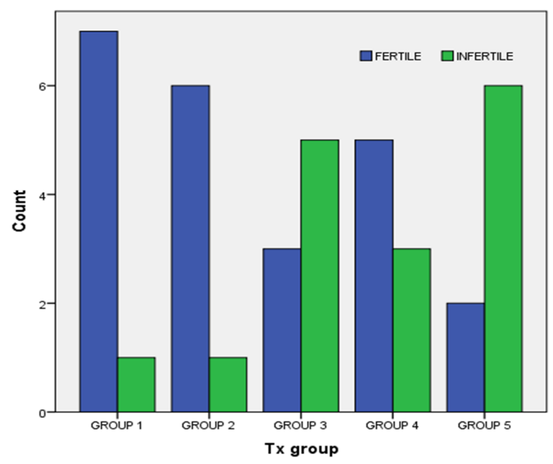
The GEE Model detected significant differences between treatment (Tx) groups and time points (P≤0.001). Post-treatment, multiple pairwise comparisons between groups highlighted significant differences in the proportion of mares maintaining potential fertility status between Group 1 and Groups 3 and 5 (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 (P≤0.005, P≤0.001,respectively) and Group 2 and Groups 3, 4 and 5 (P≤0.001, P≤0.05 and P≤0.001, respectively). Similarly, at D105 differences were detected between Group 1 and Groups 3, 4 and 5 (P≤0.05, P≤0.05 and P≤0.005, respectively) and between Group 2 and Groups 3, 4 and 5 (P≤0.005, P≤0.05 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=0.554, P=0.651, respectively).

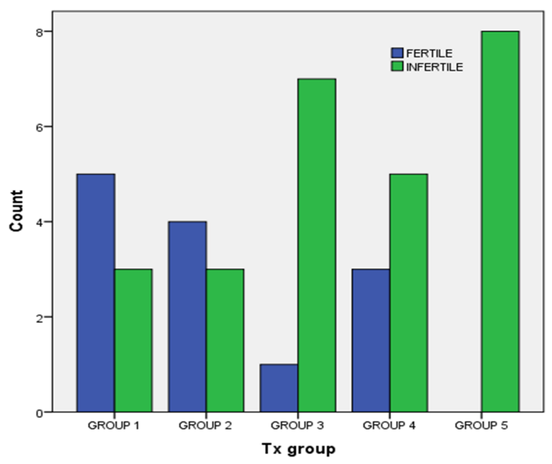
Injection site reactions, categorised as 0 (none), 1 (slight), or 2 (visible and pain) occurred in 22%, 55%, 46%, 47% and 50% of examinations in Groups 1, 2, 3, 4 and 5, respectively. Elevated rectal temperatures occurred in 20%, 20%, 26%, 9% and 17% of examinations in Groups 1, 2, 3 ,4, and 5, respectively. All reactions were mild, and transient and resolved within 7 d of treatment.

Representative trans-rectal B-mode ultrasonographic images at Day 105





Potential fertility status at D70



Potential fertility status at D105

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;
- superior with a three treatment reZP protocol compared to a two treatment pZP formulation protocol;
- similar with a three treatment reZP formulation protocol to an initial inoculation of a pZP formulation and one booster of a reZP formulation and a two treatment GnRH formulation protocol; and
- incorporation of a Pet Gel A and Poly (I: C) adjuvant formulation provided an effective alternative to currently-utilised Freund's adjuvants reportedly associated with adverse injection site reactions.

Literature cited

Barber, M.R. & Fayrer-Hosken, R.A. 2000, "Possible mechanisms of mammalian immunocontraception", *Journal of reproductive immunology,* vol. 46, no. 2, pp. 103-124.

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