

## **Effects of an anti-gonadotrophin releasing hormone vaccine on the morphology and structure of bull testes**

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Vaccination against gonadotrophin-releasing hormone (GnRH) is used as an alternative to surgical castration for the purposes of reducing pain and distress in the animals. Currently, no anti-GnRH vaccine has been authorized for use in cattle in the European Union. The aim of the present study was to assess the effect of an anti-GnRH swine-specific vaccine (Improvac<sup>®</sup>, Zoetis, USA) on the morphology and structure of bull testes.

Sixteen calves were distributed into 2 equally sized groups depending on their LW at the beginning of study: light (172.9±30.00 kg) and heavy (323.8±37.79 kg). Half of the calves in each group were randomly selected to serve as the control (C) and vaccinated (VA) group. The calves were vaccinated at days 1, 21 and 104 of the experimental period. At slaughter (day 164), testes were weighed and measured, and then tissue samples were collected and fixed in formalin. Histological and immunohistochemical studies (anti-vimentin mAb and anti-human Ki-67 mAb, Dako Denmark A/S) were performed on the testes to measure the diameter of the seminiferous tubules and assess the testicular cell populations.

Hypoplasia of the testes was found to be associated with vaccination, since the weight, diameter and perimeter measurements, and volume of the testes were lower in VA calves than in C calves ( $P < 0.0001$ ). The C and HEAVY calves exhibited higher diameters of the seminiferous tubules than the VA and LIGHT calves ( $P < 0.001$  and  $P < 0.05$  respectively).

All C calves exhibited fully developed spermatogenesis. In contrast, the testes of all the VA calves except two exhibited a complete absence of spermatogenesis with a predominance of Sertoli cells in the seminiferous epithelium. Two of the VA calves (from the HEAVY group) exhibited normal microscopic features consistent with active spermatogenesis that was similar to that described in C calves. In the VA calves except the two from the HEAVY group, the seminiferous epithelium was immunolabelled with vimentin almost in its entirety, which indicated that it was largely composed of Sertoli cells. Only a few vimentin-negative cells, which corresponded to spermatogonia, were observed in the basal seminiferous epithelium; moreover, only a few of these spermatogonia were mitotically active, as indicated by Ki-67 immunolabelling. In C and the 2 VA calves from the HEAVY group, vimentin-positive Sertoli cells were evenly distributed between the spermatogenic cells, which comprised the majority of the cells. Ki-67 antigen staining revealed the presence of a large number of active spermatogonia and spermatocytes.

Our results demonstrate that immunization of male calves against GnRH with commercially available Improvac<sup>®</sup>, which was originally developed for use in boars, severely affects testicular morphology and structure. The effect of Improvac<sup>®</sup> is more pronounced and consistent in calves vaccinated at a low LW than at a heavy LW, which suggests that vaccination is more effective when calves are vaccinated prior to puberty.